The Role of Vision and Mechanosensation In Insect Flight Control

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14. ABSTRACT
One of the main goals within this project has been to develop assays to investigate the mechanisms of visual and mechanosensory flight control in different insect species. During the course of the project, we have constructed two indoor flight facilities, equipped with light-sensitive high-speed cameras (MotionBlitz Cube EoSense) that film in the infrared spectrum of light. The use of infrared diodes in our experimental set-ups provides us with an effective tool to film insect behavior under any light level, including complete darkness. The experimental assays that we have developed have been successfully tested on bumblebees, halictid bees, flies, hornets and wasps, and we expect them to work on a large range of insects. We have repeatedly visited the lab of Sanjay Sane, at the National Centre of Biological Sciences in Bangalore, to learn how to manipulate the mechanosensory organs of flying insects and included the methods that we have learned in our experimental assays. As a result of using a wide variety of model organisms and approaches, we have made significant progress in this three-year period of the AFOSR contract in our investigations of the role of vision and mechanosensation in insect flight control. Some projects are still in progress or are nearing completion, whereas other have been completed and published in international journals. During the funding period we have accumulated a large amount of behavioral data, that has so far led to two published and one submitted paper and several others are in preparation. Our work has led to considerable understanding of how diurnal and nocturnal insects control flight visually, as well as by mechansosensation.

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MARIE DACKE AND EMILY BAIRD

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PRINCIPAL INVESTIGATORS: MARIE DACKE AND EMILY BAIRD

EXECUTIVE SUMMARY

One of the main goals within this project has been to develop assays to investigate the mechanisms of visual and mechanosensory flight control in different insect species. During the course of the project, we have constructed two indoor flight facilities, equipped with light-sensitive high-speed cameras (MotionBlitz Cube EoSense) that film in the infrared spectrum of light. The use of infrared diodes in our experimental set-ups provides us with an effective tool to film insect behavior under any light level, including complete darkness. The experimental assays that we have developed have been successfully tested on bumblebees, halictid bees, flies, hornets and wasps, and we expect them to work on a large range of insects. We have repeatedly visited the lab of Sanjay Sane, at the National Centre of Biological Sciences in Bangalore, to learn how to manipulate the mechanosensory organs of flying insects and included the methods that we have learned in our experimental assays

The experimental set-ups developed within this project have further been designed to work in a controlled lab-environment as well as in the field. The present assays thus facilitate the study of visual as well as mechanosensory mechanisms of flight control, in different insect species under different light intensities, in a whole range of conditions. The assays are described in detail below, as well as in the publications presented at the end of this report.

The methods developed within this project have, for the first time, made it possible to conduct a comparative study aimed at investigating whether the strategies of visual flight control previously observed in honeybees and fruit flies are a general feature of all flying insects, irrespective of habitat and light intensity. We used bumblebees (*Bombus terrestris*), honeybees (*Apis mellifera*), the common wasp (*Vespa vulgaris*), hornets (*Vespa crabro*) flies (*Mousca domestica*) and the nocturnal bee (*Megalopta genalis*) as our model animals. Megalopta are interesting model animals because they fly in the complex environment of the Panamanian rainforest at extremely low light intensities.

Overall, our findings demonstrate that that the visual system of diurnal, as well as nocturnal insects, rely heavily on visual information for flight speed control. This is remarkable considering the sensory challenge of controlling flight in the complex environment of a dark rainforest. The relatively low ground speed of *Megalopta* suggests that these bees use temporal summation to help them to perceive optic flow and to use it for flight control.

However, important differences between the species could also be found. Bumblebees, honeybees, flies and wasps try to balance the rate of visual motion experienced in each eye when flying along the experimental tunnel. This strategy ensures that the insects maintain an equal distance to nearby obstacles on their left and right sides (i.e. that they fly through the centre of holes). *Megalopta*, rather surprisingly, has developed a different strategy for avoiding nearby obstacles. This novel and so far unknown method for collision avoidance in a dark and complex environment will be further investigated in May 2012.

From the studies of our main experimental animal – the bumblebees – we could also conclude that bumblebees respond to changes in the rate of axial (front-to-back) optic flow within a frontal visual field that lies approximately between 23 and 30° from the midline – not in the more lateral visual regions. It appears as though the bees are not simply averaging the rate of optic flow experienced across the entire visual field to regulate flight speed. Instead, it seems that they only pay attention to the higher rates of

optic flow – generated by nearby obstacles – present in the visual field. Studies are currently being undertaken to investigate if this is indeed the case. Comparative studies on our other model insects are planned to test for the generality of these findings.

Although it is useful to perform experiments using simplified stimuli (such as the black and white pattern presented to the insects in the studies above), it is also important to consider how an animal uses the visual information that it encounters in its natural environment. How well do the results from studies with a simplified environment reflect the natural behaviour of flying insects, considering the radical differences between these two classes of visual environment? We have aimed to answer this question by investigating how bumblebees regulate their groundspeed in an outdoor setting using three- and two-dimensional naturalistic scenes as well as two-dimensional artificial scenes. Overall, our results suggest that groundspeed is not affected by whether the visual scene is naturalistic or artificial, or whether the experiment is conducted indoors or outdoors. These findings have implications not only for understanding groundspeed control in bumblebees, but also for the results of past and future investigations into visually guided flight control in other insects.

Together with our collaborator Dr. Sanjay Sane from the National Centre for Biological Sciences (Bangalore, India), we have measured inter-antennal angles of free flying bumblebees in response to varying ground speeds. Interestingly, these results reveal a significant negative correlation between ground speed and inter antennal angle. Because the antennae are brought forward with increasing air speed (against the force of drag) the antennae appear to respond actively to the speed of airflow. The function of this enigmatic antennal response remains to be investigated.

The antennae provide crucial mechanosensory cues for flight control in moths. When the distal parts of the antennae are removed, the moth is able to fly but it is unable to control its flight path. To investigate if the antennae play a similar role for flight stabilization in bumblebees, bees with intact or manipulated antennae were filmed flying down an experimental tunnel displaying strong visual cues. Our preliminary results points towards that antennectomized bees fly slower and with a larger lateral movement in their flight path than untreated bees (figure). This suggests that the antennae play a role for flight stabilization also in the bumblebee. So far, we only have a limited data-set from 3 different bees in each condition, but a growing set of data is currently being collected.

This final report of our project grant is a summary of the progress made within the three years scope of this project. The work from the last 6-month project period of this grant is included in this report.

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I. GENERAL INTRODUCTION

In flying organisms such as insects, the sensory modalities that are available for flight control and navigation are more constrained than is the case in man-made aircraft. Insects do not carry radio communications equipment, radar, GPS, infrared sensors or large precision inertial systems, but rather get by with an assembly of conventional senses such as vision, mechanoreception, hearing and chemoreception. However, this sensor assembly, together with the information processing circuitry of the insect brain, is extremely miniaturized in comparison to any existing technical systems. Furthermore, each of these sensory systems has been under evolutionary selective pressure for the optimization of its sensitivity and acuity.

Despite the limitations of their small brains and relatively simple sensory systems, flying insects demonstrate the remarkable ability to fly and navigate under a wide range of ambient light intensities, covering more that 8 orders of magnitude between full sunlight and the night sky. Some recent work from our research group in Lund is starting to show that low-light flight and navigation occurs at intensities that are well below what was previously thought to be possible (Dacke et al., 2004, 2003; Greiner et al., 2005; Kelber et al., 2002; Theobald et al., 2007; Warrant, 2004; Warrant et al., 2004). Nocturnal insects can see color and negotiate dimly illuminated obstacles during flight. They can also navigate using learned terrestrial landmarks, the constellations of stars or the dim pattern of polarized light formed around the moon. To control flight and avoid obstacles at low light levels, flying insects must be able to quickly and efficiently integrate useful information from a number of sensory modalities, particularly vision and mechanosensation.

The insect's dependence on multiple sensory inputs for flight control poses significant challenges especially during the fast maneuvers that are necessary for the insect to remain airborne and to avoid collisions. One such challenge involves integrating and processing the sensory feedback from various modalities as it arrives in the brain with varying latencies. For instance, mechanosensory information is usually transduced rapidly (< 5-10 ms; Dieudonne et al, *in prep*) via fewer interneurons and larger axons, whereas visual information is slower (> 20 ms; Warzecha and Egelhaaf, 2000) owing to a longer transduction cascade. Thus, although the input from mechanosensory modalities is available to the insect well in advance of the next wing stroke, the simultaneous visual input may not be available until a few strokes later. Under low light levels, the visual-mechanosensory latency difference is especially great because the eye must wait longer to form or update an image (Warrant, 1999). This finding has been confirmed behaviorally by Theobald et al. (2007), who recently showed that flight performance in the nocturnal bee *Megalopta* decreases with decreasing light intensity. Insects flying at low light intensities may thus find it harder to use visual input to stabilize flight on a wing stroke-to-wing stroke basis.

I.1. The role of visual information in insect flight

Diurnal insects such as flies and honeybees rely heavily on vision to control flight (e.g. David, 1979; Egalhaaf and Borst, 1993; Srinivasan et al., 1996). For instance, it is well-known that a wide-field looming stimulus, signaling the approach of a large object presented in front of tethered flies elicits a 'leg extension reflex' - a stereotypic response in which the front legs are held forward in anticipation of landing (Goodman, 1960; Borst and Bahde, 1986; 1988). On the other hand, the same stimulus presented on one side of the insect elicits a collision avoidance response resulting in rapid turns away from the stimulus (Tammero and Dickinson, 2002). Similarly, looming stimuli elicits take-off

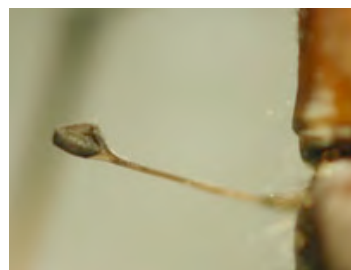response in resting insects, perhaps because they signal approaching danger (Card and Dickinson, 2008).

Visually mediated behavioral responses are especially relevant to flying insects. For example, flying insects are often required to navigate through cluttered environments (e.g. dense foliage of trees) where the potential for collisions with obstacles is high. In such environments, they must control their flight speed to enable their sensory systems to detect and avoid obstacles. Previous research demonstrates that honeybees and flies use visual cues to regulate their flight speed in cluttered environments (Baird et al., 2005; Srinivasan et al., 1996; David, 1982) and to adjust their speed prior to landing (Baird et al., *in prep,* Srinivasan et al., 2000). When the distance to nearby surfaces decreases, these insects perform compensatory decreases in flight speed. Interestingly, these studies have been conducted on diurnal insects under relatively high light intensities, when the transduction time of visual information for these animal is at its fastest. Although visual information has been shown to be useful for flight speed control at high light intensities, can insects have such a heavy reliance on visual cues to regulate flight in dim light, when the latency of the visual feedback is increased?

I.2. The role of mechanosensors in insect flight

The mechanosensory feedback required for flight control is obtained from different organs in different insect species. In two-winged insects, the hind pair of wings have evolved into mechanosensory structures called halteres which are crucial in active flight control (Pringle, 1948; Nalbach, 1993). In four-winged insects such as moths and butterflies, which lack halteres, critical feedback for flight control is obtained from the antennal mechanosensors (Sane et al. 2007). As in the case of halteres, the proper mechanical loading of the basal mechanosensors of antennae is crucial for flight stability in the sphingid moth, *Manduca sexta*. Thus, when the distal part of the antennae are removed, thereby depriving the basal mechanosensors of the normal input, the moth is able to fly but it is unable to control its flight path. When the antennae are reattached, the moth regains its ability to control flight. These experiments show that antennae provide crucial mechanosensory cues for flight control.

Halters in flies (top) and antennae in butterflies (bottom) are two structures that can provide critical mechanosensory feedback for flight

Because *Manduca* are crepuscular, the experiments described above were conducted under dimly lit conditions. In dim light, the slower visual processing meant that the insects had to depend on the rapid mechanosensory feedback from their antennae. Is rapid mechanosensory feedback equally necessary under bright light levels when visual feedback is faster? How do insects use both mechanosensory and visual feedback in conditions of varying mechanical and visual processing times?

Insect eyes vary greatly in their spatial acuity and response latencies. Similarly, the mechanosensory structures also exhibit morphological diversity. Because these differences cause a corresponding variation in signal transduction, the relative importance of vision and mechanosensation may also show substantial variation from one insect group to another, or even within the same individual under variable light conditions.

To study the individual and combined contributions of vision and mechanosensation in a composite stimulus situation, we need to develop flight assays that enable us to manipulate the gains of visual and mechanonsensory feedback and to observe the effect on fundamental flight control behaviors such as flight speed and height control and obstacle avoidance. How does an insect process two or more sensory inputs with different latencies during flight? How do these latencies influence the time it takes for an insect to respond to each stimulus? The answers to these questions are crucial for gaining a better understanding of the role of sensorimotor mechanisms in insect flight control. The mechanosensory component of this study is conducted in collaboration with Dr. Sanjay Sane and his group at the National Centre for Biological Sciences in Bangalore, India.

II. Role of Vision in Flight Control

II.1. Effect of changes in surface proximity on flight control

Previous work has shown that *Drosophila* and honeybees use visual cues to regulate their flight speed when flying along experimental tunnels. They do this by holding constant the apparent speed of the visual environment. One notable consequence of this flight speed control strategy is that flight speed will be inversely proportional to the distance to surfaces in the environment (walls in a tunnel for example). This is because, for a given ground speed, the apparent rate of optic flow on the eye will be greater for an insect flying along a narrow tunnel – resulting in a decreased flight speed – than in a wider one. As such, these insects would fly slower in a narrow tunnel (where the apparent rate of optic flow would be higher) than in a wider tunnel (where the apparent rate of optic flow would be lower). Do bumblebees exhibit a similar flight control strategy to that of honeybees and *Drosophia*?

To answer these questions, we trained bumblebees (*Bombus terrestris*, fig. 1) to fly to a feeder placed at the end of an experimental tunnel. We filmed the flights of the bees in the tunnel from a camera mounted above the tunnel's central section.

In the first experimental condition, the walls of the tunnel were lined with a random texture and the walls were set either 15 or 30 cm apart (fig. 2). In each of the 15 cm and 30 cm wide tunnels, the bumblebees flew at a relatively constant forward speed. However, the bumblebees flew faster in the 30 cm wide tunnel than in the 15 cm wide tunnel (fig. 3). This result is what we would expect if the bees were relying on optic flow from the tunnel walls to regulate their flight speed. The results of this experiment were published in Baird et al. (2010)

Next, we investigated how *changes* in the proximity of nearby surfaces affect flight speed control in bumblebees and wasps. To do this, we recorded
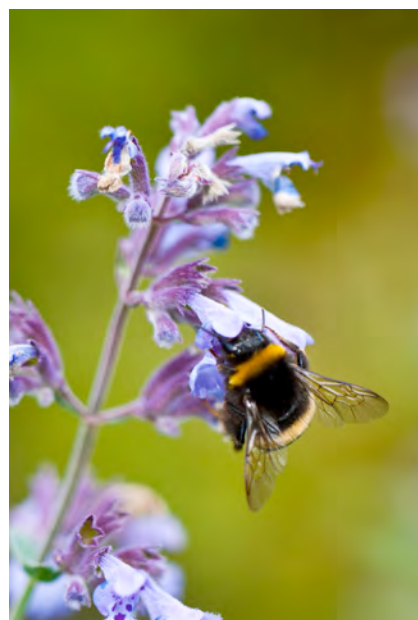

Fig. 1: The main model species for this project, the bumblebee *Bombus terrestris*.

Fig. 2: An indoor flight facility. A bumblebee hive is placed at the far left corner of the cage.

flight trajectories of bumblebees flying in an experimental tunnel whose width changed abruptly, halfway along its length. Our results showed that bumblebees slow down, or speed up in response to a sudden change in tunnel width some distance before they actually fly past it (fig. 4). According to our calculations, the change in ground speed takes place when the change in tunnel width occupies a visual angle of approximately 28° in a tunnel whose width changes from 15 to 30 cm and 30° in a tunnel which changes width from 30 to 15 cm. Interestingly, when the tunnel walls remained straight but the pattern changed from chequerboard to axial stripes, the change in ground speed took place when the change in pattern occupied a smaller visual angle of approximately 23°. Overall, this result indicates that bumblebees respond to changes in the rate of front-to-back optic flow within a frontal visual field that lies approximately between 23 and 30° from the midline – not in the more lateral visual regions. The results of this experiment were published in Baird et al. (2010).
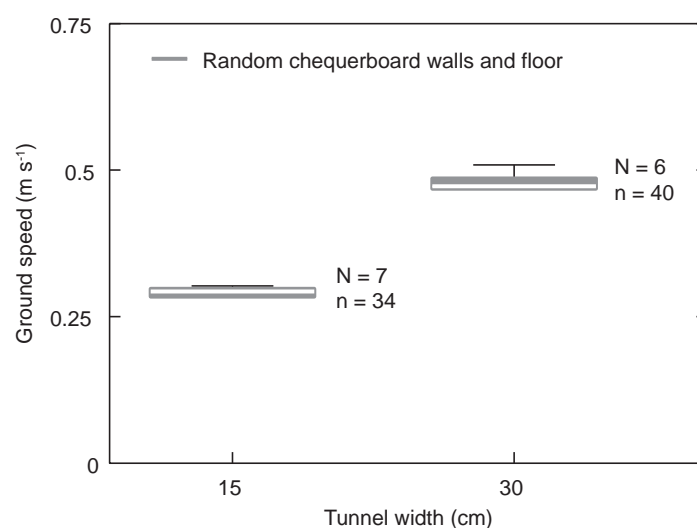

Fig. 3: The effect of tunnel width on ground speed in bumblebees. Boxes indicate the distance between the lower and upper quartile values, white lines indicate the median values and whiskers indicate the entire spread of the data. N represents the number of bees, n represents the number of flights.
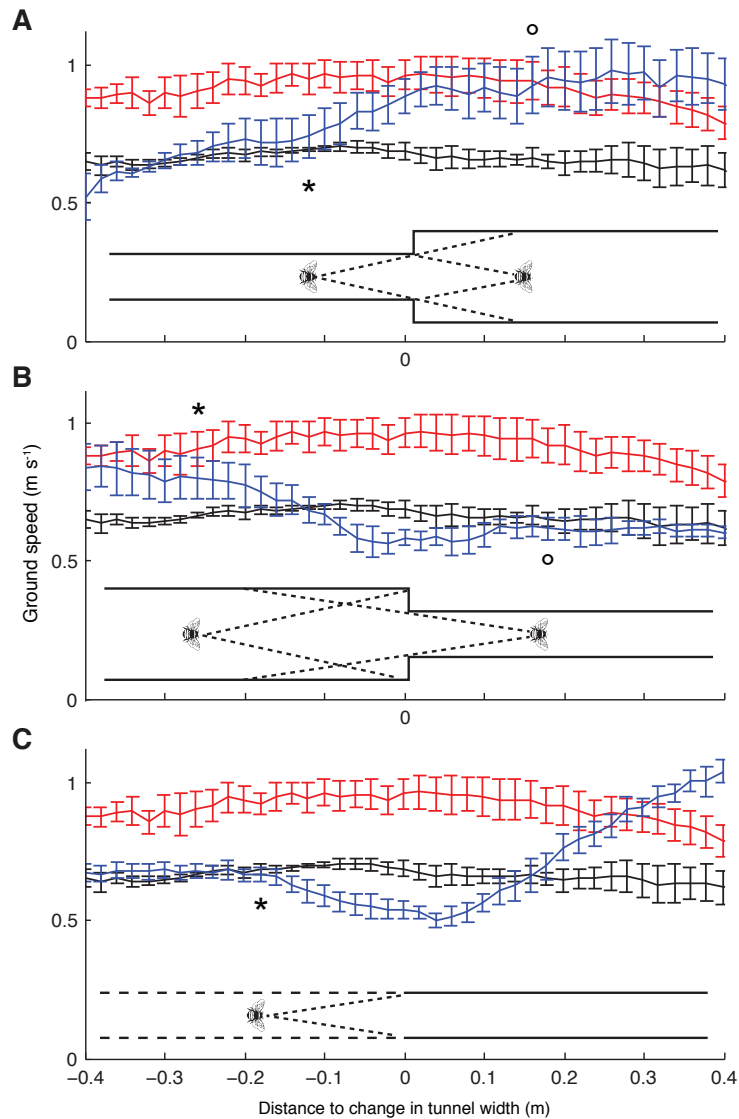
Fig. 4: Effect of abrupt changes in tunnel width on ground speed. Effect of abrupt changes in axial optic flow on ground speed (blue lines) when the width of the tunnel changes from 15 to 30 cm (A), from 30 to 15 cm (B) or when the pattern in a 15 cm wide constant width tunnel changes from random chequerboard to axial stripes (C). Black lines represent the mean ground speed of bumblebees flying in the 15 cm wide constant width tunnel, and red lines represent the mean ground speed of bumblebees flying in the 30 cm wide constant width tunnel. Means are calculated over 2 cm bins, error bars represent the standard deviation of data within each 2 cm bin (see Methods). Stars represent the position at which the test condition (blue lines) deviates significantly from the control condition (15 cm constant width for A and C; 30 cm constant width for B). Circles represent the position at which the test condition first reaches the level of the control condition in the second half of the tunnel (30 cm constant width for A; 15 cm constant width for B). We recorded 45 flights from 11 bees in the 30 cm control condition, 56 flights from 9 bees in the 15 cm control condition, 57 flights from 19 bees in the 15 to 30 cm condition (A), 35 flights from 8 bees in the 30 to 15 cm condition (B) and 30 flights from 11 bees in the chequerboard to axial condition (C).

II.2. Effect of visual texture on flight control

Although previous work has shown that honeybees and Drosophila use visual cues to regulate their flight speed, the importance of vision for flight control in other insects remained unclear. To understand the effect of light intensity on flight control in the bumblebee, it was therefore necessary to begin our study with an investigation that characterised
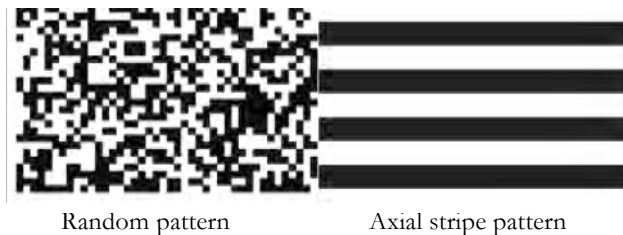

Random pattern   Axial stripe pattern

Fig. 5: The two different patterns, axial and random, that were placed on the walls and floor of the experimental tunnel

the role of vision in flight control in these insects. We started by comparing the flight speed of bumblebees flying in the experimental tunnel when the walls displayed visual cues that would either generate strong or weak front-to-back visual motion cues (randomised chequerboard pattern or horizontal stripe pattern, respectively; fig. 5). We found that bumblebees fly faster when the tunnel walls generated weak visual motion cues (horizontal stripes), than when the walls generated strong visual motion cues (randomised chequerboard pattern) (fig. 6). Overall, these results indicate that bumblebees, like honeybees and Drosophila rely heavily on visual cues to control flight.
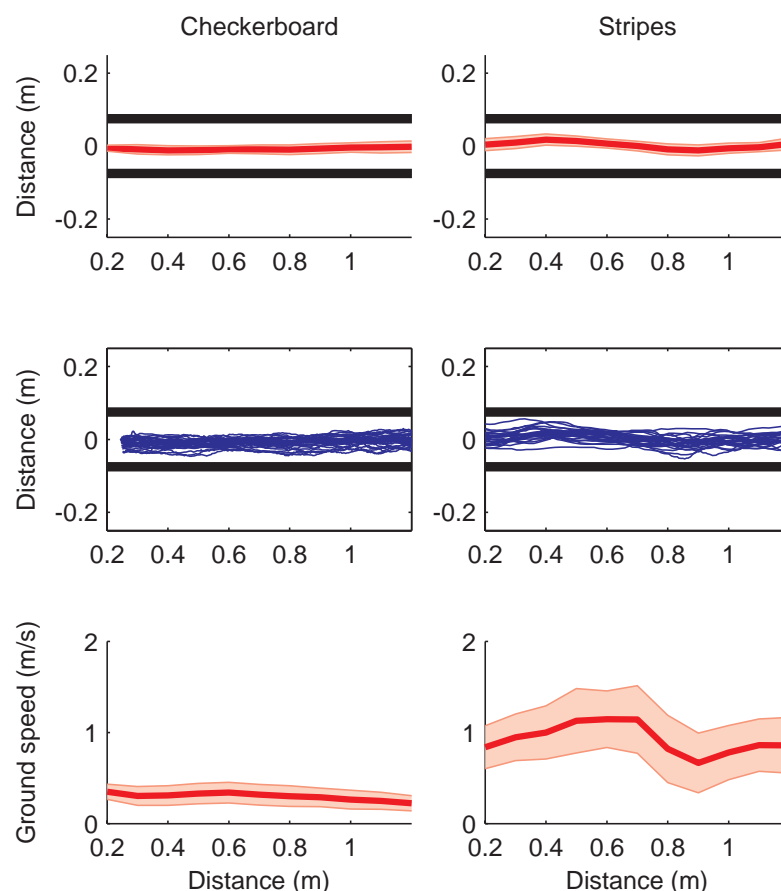
Fig. 6: The effect of visual texture on flight speed in bumblebees. Flights were recorded from above when a 15 cm wide flight tunnel was lined with either a random checkerboard pattern (generating strong optic flow cues, left column) or a horizontal stripe pattern (generating weak optic flow cues, right column). The top row shows the mean position and standard deviation of flights in the tunnel. The second row shows individual flight trajectories. The third row shows the mean (dark red lines) and the standard deviation of ground speed at each point in the

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Fig. 7: The common wasp, *Vespa vulgaris*.

To investigate whether the strategies of visual flight control that we had observed in bumblebees were a general feature of all flying insects, we repeated the experiments that we had performed on bumblebees on the common wasp (*Vespa vulgaris*) and the nocturnal bee (*Megalopta genalis*). Wasps (fig. 7) and *Megalopta* (fig. 8) are interesting model animals because they fly at lower light intensities than the day-active insects that have typically been used for visual flight control investigations. *Megalopta* are especially interesting because in the complex environment of the Panamanian rainforest at extremely low light intensities. Our results showed that, despite the difference in their natural habitats and behavioural requirements, wasps (fig. 9) and *Megalopta* (fig. 10) both rely heavily on visual information for flight control.

Fig. 8: The nocturnal sweat bee, *Megalopta genalis*.
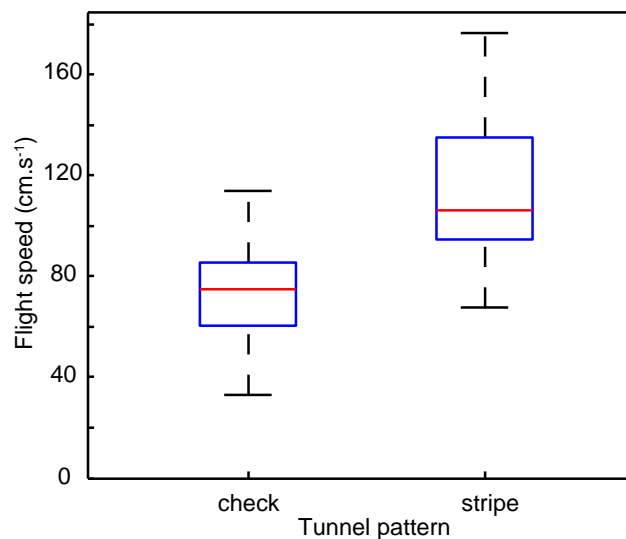
Fig. 9: The effect of visual texture on flight speed (ground speed) in the common wasp, *Vespa vulgaris*.
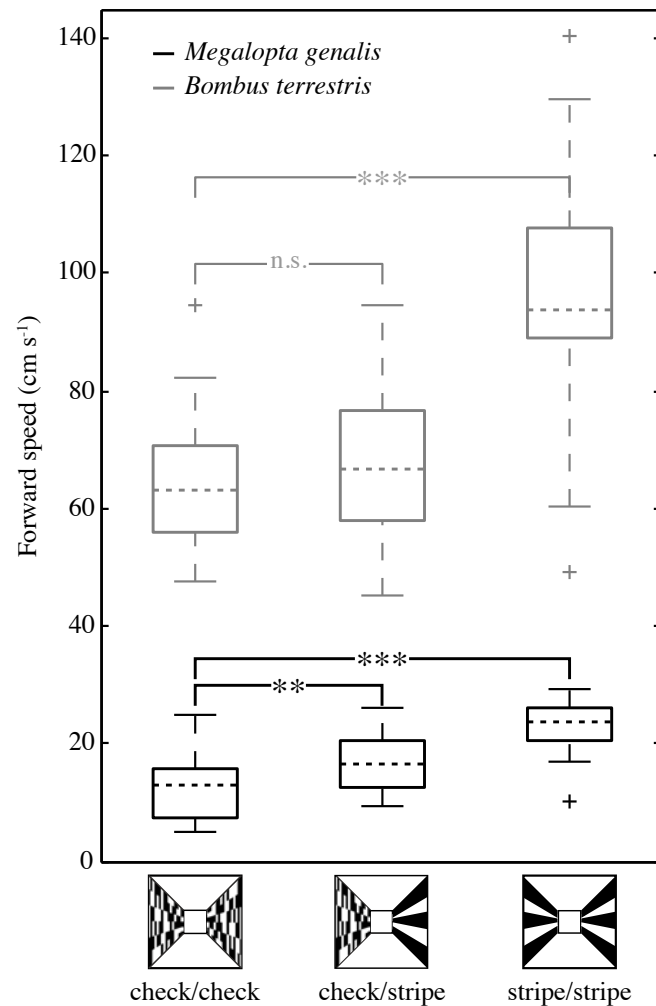
Fig. 10: The effect of changes in horizontal optic flow cues in the tunnel on the groundspeed of *Megalopta genalis* (*black boxes*) and *Bombus terrestris* (*grey boxes*). Significance codes – **; $p <$ 0.01, ***; $p <$ 0.001, n.s.; not significant. Box limits represent the 25th and 75th percentiles of the data, *dotted lines* indicate the median, *whiskers* extend to the rest of the data, *crosses* indicate outliers. Both species increase their groundspeed when horizontal motion cues are minimised, but unlike *Megalopta*, bumblebees do not fly faster when horizontal motion cues are removed from one wall.

Our investigations with *Megalopta* also revealed that, although they fly faster when visual motion cues are minimised – as we had previously observed in bumblebees – they do not use the same strategy for centring (fig. 11). When the walls of a tunnel generate strong visual cues, both bumblebees and *Megalopta* tend to fly along the midline of the tunnel. If the visual motion cues generated by one wall are reduced, bumblebees fly closer to the wall that generates less visual motion. This suggests that the bumblebees try to balance the rate of visual motion experienced in each eye when flying along the tunnel, a strategy that would ensure that they maintain an equal distance to nearby obstacles on their left and right sides (i.e. that they fly through the centre of holes). *Megalopta*, rather surprisingly, did not react in the same way when the visual motion cues on one wall of the tunnel were reduced. In this case, *Megalopta* still flew along the midline of the tunnel. It therefore appears as though Megalopta has developed a different strategy for avoiding nearby obstacles. This is perhaps because the majority of visual information in the habitat of Megalopta comes from above, where the dark canopy makes a high contrast pattern against the relatively bright sky. The results of this study were published in Baird et al. (2011).
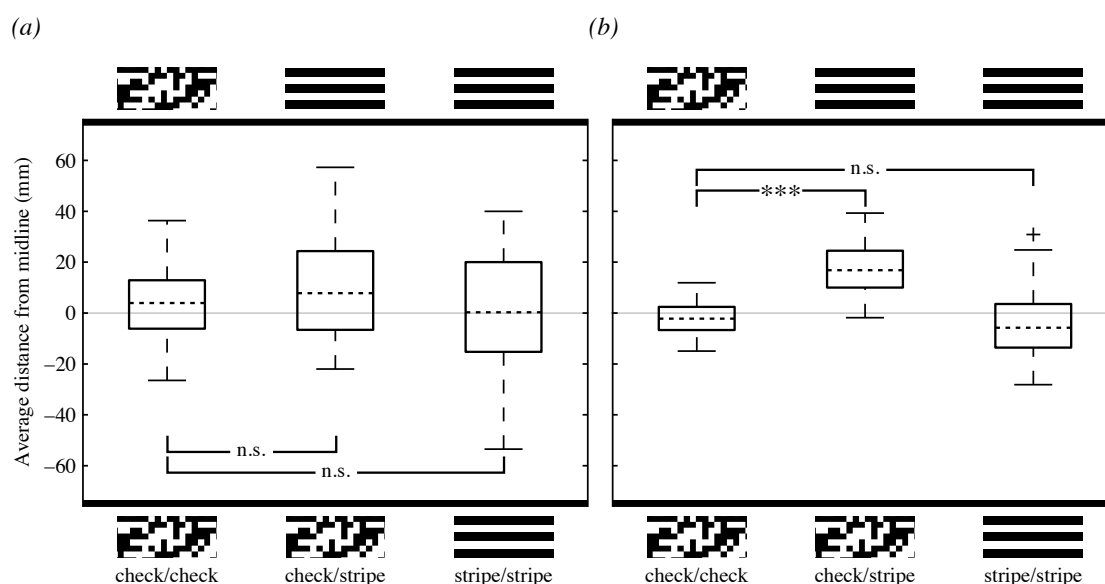
Fig. 11: The effect of changes in horizontal optic flow cues on centring in *Megalopta genalis* (*a*) in *Bombus terrestris* (*b*). *Thick black lines* indicate the tunnel walls; *light grey lines* indicate the midline of the tunnel and the *pattern (check or stripe)* indicates the position of the patterns. Other details as in figure 1. In the check/stripe condition, bumblebees fly closer to the stripe pattern; we see no such effect in *Megalopta*.

II.3. Effect of naturalistic 3D stimuli on flight control

Studies investigating how insects use optic flow to control flight (including our own investigations) have typically been conducted under relatively controlled laboratory conditions and the visual environment of the insects has been restricted to relatively simple, artificial stimuli such as two-dimensional gratings and chequerboards. This approach is effective because it enables experimenters to precisely control features of the external environment (such as temperature and light intensity), to manipulate different features of the visual environment (such as contrast and spatial frequency), and to easily record the behavioural response. One of the major limitations of this approach is that

the conditions under which these experiments are conducted, and the simplistic visual stimuli that are used, bear little resemblance to the highly complex and dynamic natural visual environment of the insect. Although it is useful to perform experiments using simplified stimuli, it is also important to consider how an animal uses the visual information that it encounters in its natural environment.

In order to really understand how flying insects are using vision to control flight at different light intensities, we need to be sure that they are responding to the visual stimuli that we are using (randomised black and white checkerboards, for example) in the same way as they respond to visual information in their natural environment. Therefore, we performed an investigation into how bumblebees regulate their ground speed in an outdoor setting using three-dimensional and two-dimensional natural scenes and to compare the results with the results obtained from similar experiments conducted in the laboratory using simple visual stimuli. We began by using a natural scene and an artificially generated pattern (randomized checkerboard) to examine the effect of these two different stimuli on ground speed control. Next, we used 2D and 3D naturalistic visual stimuli to investigate how ground speed is affected by the dimensionality of the visual stimulus. Finally, we tested the effect of changes in proximity to visual stimuli on ground speed control when the stimuli are either 2D or 3D (fig. 12).
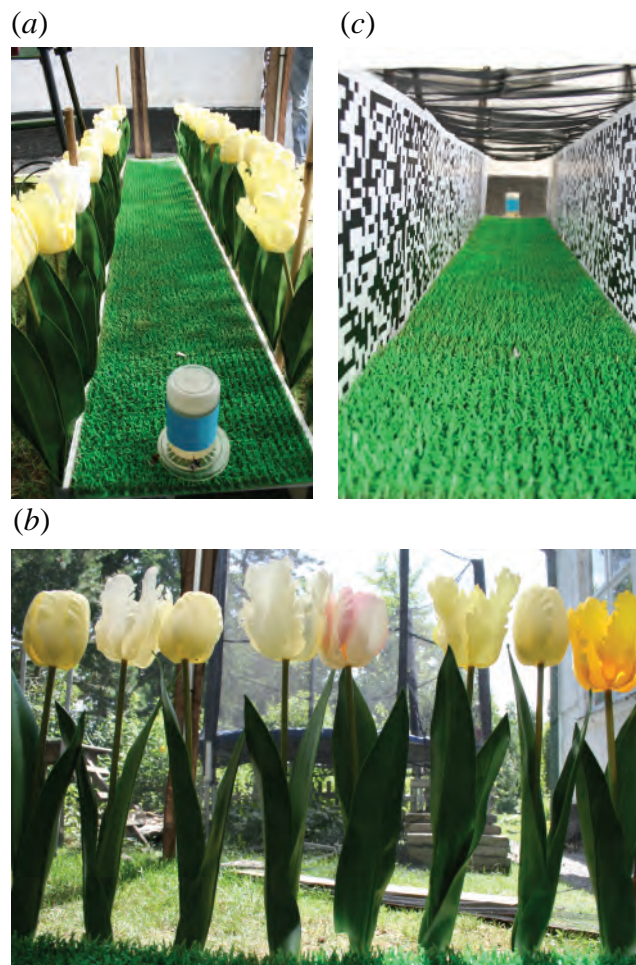
Fig. 12: Visual stimuli used to investigate the effect of natural visual stimuli on flight control. (*a*) An image taken looking along the length of the experimental tunnel from the feeder to the entrance, when the walls are made up of plastic tulips ('naturalistic scene'). (*b*) A part of the 'naturalistic scene' and an example of the images used to make up the 'naturalistic picture' visual stimulus (see Methods for details). (*c*) A view along the experimental tunnel from the entrance to the feeder (marked with blue) when the tunnel walls were lined with the artificial pattern.

The results of our experiments (fig.13) suggest that ground speed is not affected by changes in the type of visual scene available (natural or artificial) or its dimensionality. When the visual stimulus is either a two-dimensional or three-dimensional naturalistic scene, bumblebees adjust their ground speed according to the distance of nearby surfaces. These results are consistent with those of the experiments we performed using artificial stimuli in a controlled indoor environment. Therefore, it appears that the flight control behaviours observed under controlled conditions using simplistic visual stimuli are a

close representation of the natural behaviour of bumblebees. These findings have implications not only for understanding ground speed control in bumblebees, but also for the results of past and future investigations into visually guided flight control in other insects. The results of this study have been submitted to the journal PLoS One.

Fig. 13: The effect of naturalistic and artificial visual stimuli on flight control in bumblebees. The groundspeed of bumbl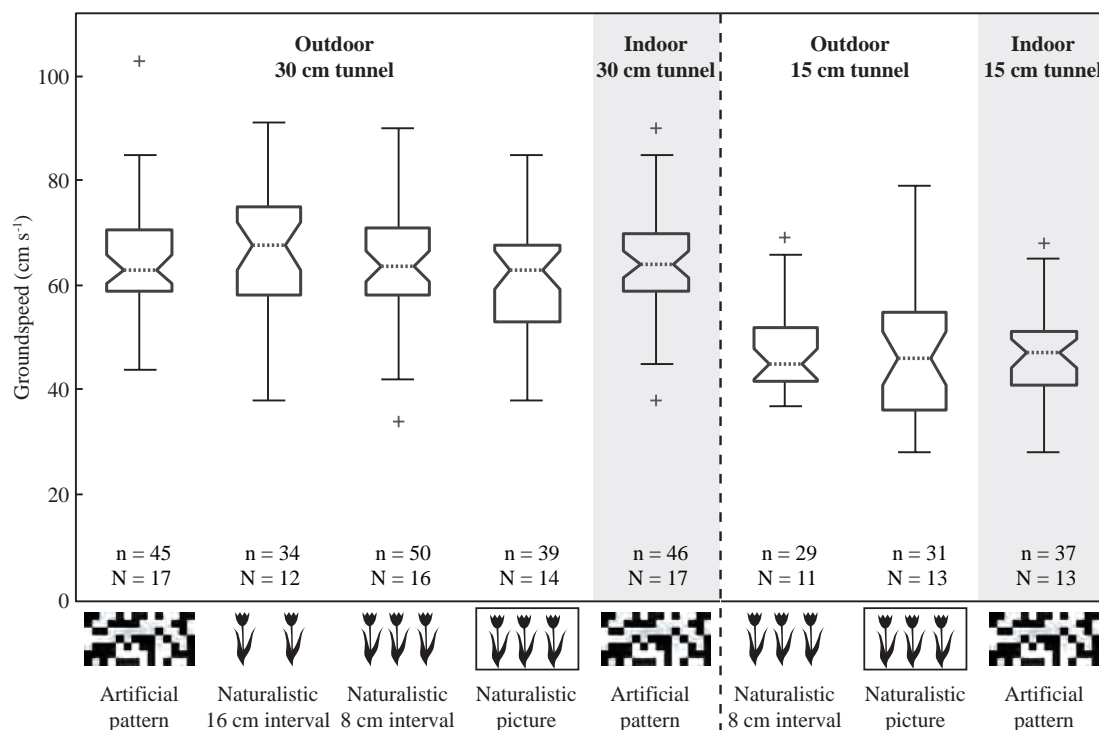ebees flying along experimental tunnels with walls made up of different visual stimuli (see Methods for details). The distance between the walls was either 30 cm or 15 cm and the experiments were conducted either outdoors or indoors; n indicates the number of flights in each condition, N indicates the number of bees. The dotted line marks the median of the data, the boxes extend between the 25th and 75th percentiles, notches indicate the 95% confidence interval, whiskers indicate the rest of the non-outlier data and crosses indicate outliers.

The results of the experiments described above suggested that, in a 3D environment, bumblebees regulate their flight speed based on the rate of optic flow generated by only nearby obstacles in the visual field. In other words, it appeared as though the bees were not simply averaging the rate of optic flow experienced across the entire visual field to regulate flight speed. Instead, it seemed that they only pay attention to the higher rates of optic flow – generated by nearby obstacles – present in the visual field. To investigate if this is indeed the case, we trained bees to fly along an experimental tunnel, which consisted of nearby obstacles (vertical poles displaying a check pattern) set against a distant background of a vertical wall also displaying a check pattern (fig. 14). To change the average amount of optic flow experienced by a bee flying along the tunnel, we varied both the distance between the poles and the distance to the background wall (and indeed whether this wall was present or absent). By varying the distance between the poles, we were able to change the amount of 'background' optic flow experienced by the bees. The bees will see more of the slower moving background as the distance between the faster moving near obstacles increases, leading to a reduced average optic flow estimation. By varying the distance between the walls, we can also change the average optic flow experienced by bees flying along the tunnel, because the background will generate a lower rate of optic flow as the distance to the walls increases. If the bees are using an

average optic flow estimate to control flight speed, then we expect flight speed to increase as the distance between the poles increases i.e. as the amount of nearby 'fast' optic flow in the visual field decreases and the amount of far 'slow' optic flow increases. On the other hand, if the bees are only measuring the fast nearby optic flow for flight speed control, the changes in the distance to the background will not affect flight speed. The data from these experiments are currently being analysed and prepared for publication.
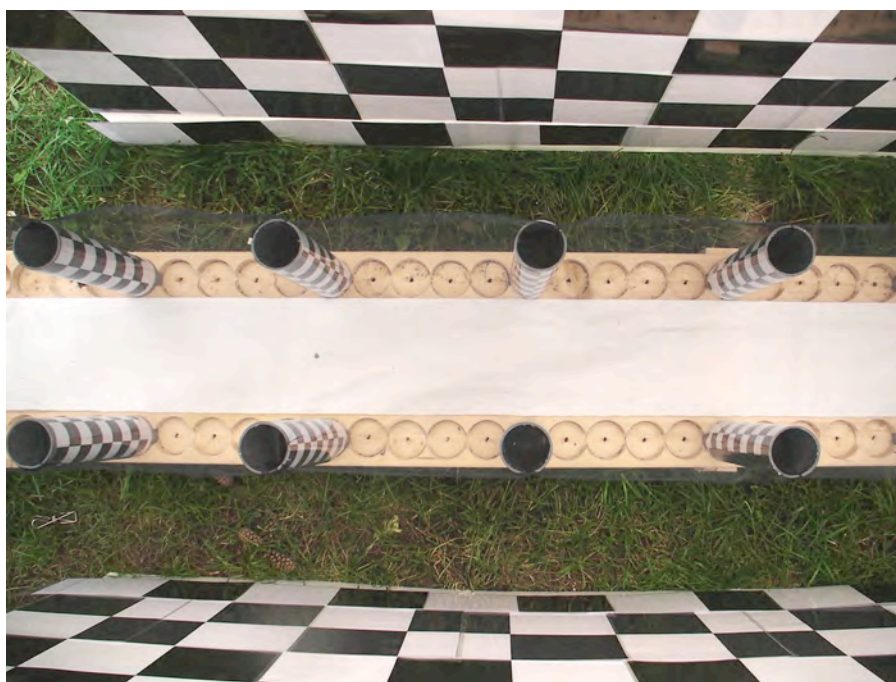

Fig. 14: The experimental set-up used to investigate flight control in three-dimensional environments. By varying the distance between the vertical poles it is possible to vary the amount of nearby optic flow experienced by bumblebees flying to a feeder at the end of the tunnel. Walls were used to provide background optic flow.

II.4. Effect of light intensity on flight control

Interestingly, former studies of flight control have all been conducted on diurnal insects under relatively high light intensities, when the transduction time of visual information for these animal is at its fastest. The aim of this section of the project is to identify the range of light levels over which an insect can use visual information to control flight, and how mechanisms of flight control are affected by changing light levels.

Activity data is an important indication of the range of light levels over which the insects can use visual (and mechanosensory) input to control flight. To gain a close understanding of the activity patterns of our model insects, we have filmed and later quantified the number of bumblebees (*Bombus terrestis*) flying in or out of the hive, as well as the number of bees visiting the artificial feeder inside the flight facility. All measurements were implemented within 15-minute intervals. We find that the activity of *Bombus terrestris* peaks in morning, approximately one to two hours after sunrise, but does not cease until 45 minutes after sunset. These measurements indicate that bumblebees are not only active after dusk, but are also able to successfully forage and fly under these dim light conditions, even in the artificial environment of the lab. We are currently in the process of developing experimental equipment that will allow us to record the flight

activity of bumblebees when they are housed outside under more natural conditions. This will then allow us to investigate the effect of light intensity on flight speed in a more natural environment. This work is scheduled to commence in Spring 2012, when the weather will permit us to perform outdoor experiments.

The nocturnal tropical sweat bee, *Megalopta genalis*, flies at much lower light intensities than most hymenopterans. This makes *Megalopta* an ideal model species for investigating the effect of light intensity on flight control. To do this, we placed flight tunnels in front of the nests of Megalopta and filmed the flights when the bees returned to their nests after a foraging trip. Our results indicated that *Megalopta* tends to fly slower as light intensity decreases (fig. 15). This result is interesting because it supports the hypothesis put forward by Warrant (1999) that *Megalopta* use temporal summation to be able to see in dim light.
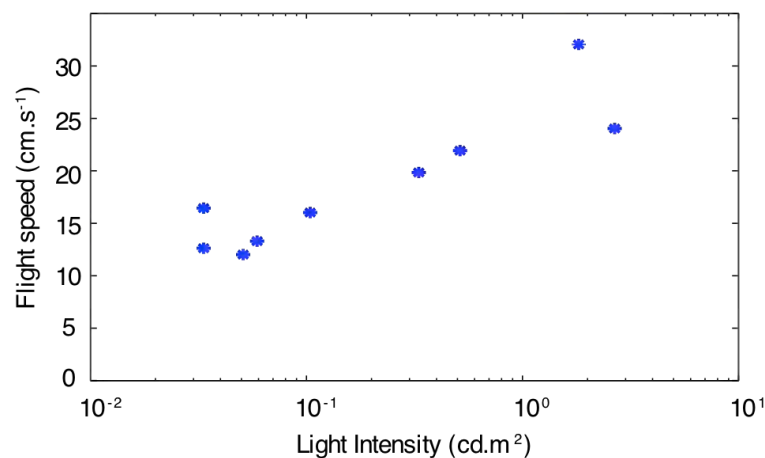
Fig. 15: The effect of light intensity on flight control in *Megalopta*. The flight speed of *Megalopta* over light intensities that span four orders of magnitude.

We continued our investigations of the effect of light intensity on flight control in *Megalopta* by exploring how light intensity affected the accuracy of landing. To do this, we filmed *Megalopta* landing on their nest sticks (and artificial disks that we placed on them) under a broad range of light intensities (fig. 16). To determine how 'accurately' the bees landed under the different light conditions, we analysed the leg extension reflex, a stereotyped behaviour that the bees perform just before they land. To analyse the data,
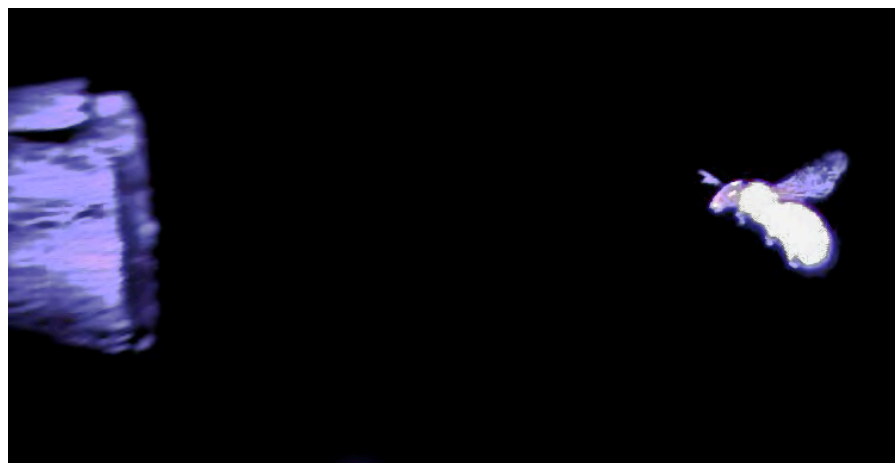
Fig. 16: A *Megalopta* bee coming in to land on her nest stick. We investigated the accuracy of landing in *Megalopta* over different light intensities.

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we calculated both the distance between the bee and the nest when leg extension occurred and also the 'time-to-contact (i.e. the time between leg extension and contact with the nest). We would expect that *Megalopta* would perform the leg extension reflex closer to the nest in dim light because it is more difficult to see. Our results revealed a surprising consistency in the time before contact at which the leg extension reflex was initiated, even across a broad range of light intensities (fig. 17). This suggests that *Megalopta* are as accurate at landing under dim light conditions as they are at landing under bright light.
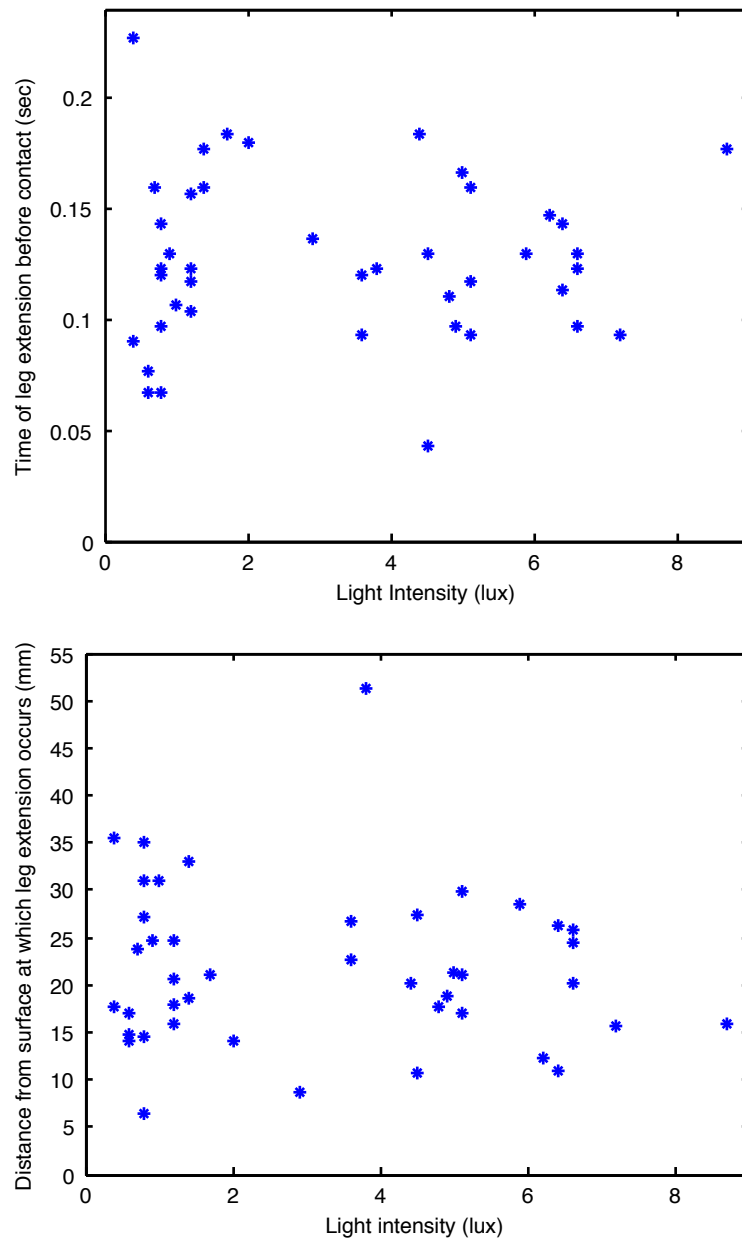
Fig. 17: The effect of light intensity on landing accuracy in *Megalopta*. The time between leg extension and landing (top) and the distance from the landing surface at which leg extension occurs (bottom) over a range of light intensities.

II.5. Obstacle avoidance

The nocturnal tropical sweat bee, *Megalopta genalis*, is active under very dim light conditions. This is surprising given that it's eye morphology is not especially adapted to the high sensitivity that is required for seeing motion in dim light. It is possible that these bees overcome the limitations of their visual system by developing a greater reliance on mechanosensory cues to detect and avoid obstacles in dim light. These cues could come from the air vibrations that rebound off an obstacle when the bee is nearby, or from the air-flow that is deflected off the obstacle and back onto the bee.

To test this whether *Megalopta* do indeed rely on mechanosensory cues to detect obstacles in dim light, we placed a piece of glass in front of a nest stick after a bee had left for a foraging trip (fig. 18). The glass was specially designed to be non-reflective and to pass all wavelengths of light so that it was effectively invisible to the bees. We then filmed the bees using a high-speed camera so that the fine detail of the behavioural response to the glass could be recorded.

Fig. 18: Obstacle avoidance in dim light. A *Megalopta* bee approaching a glass disk placed in front of her nest (far left). The bee in this image did not detect the disk and crashed into it as a result.

The only way that *Megalopta* could detect the piece of glass would be if they used mechanosensory cues. Our high-speed recordings showed that, in many cases, the bees performed emergency landing manoeuvres only when they had come into physical contact with the glass (i.e. touched it with their antennae). In a few cases, the bees actually crashed into the glass. These results suggest that *Megalopta* has not developed a greater reliability on mechanosensory cues for detecting obstacles when flying in dim light.

III. ROLE OF MECHANOSENSATION IN FLIGHT CONTROL

III.1. Role of antennal mechanosensors for flight control

In four-winged insects such as moths and butterflies, critical feedback for flight control is obtained from the antennal mechanosensors. The proper mechanical loading of the basal mechanosensors of antennae is crucial for flight stability. Thus, when the distal part of the antennae are removed, thereby depriving the basal mechanosensors of the normal input, the moth is able to fly but it is unable to control its flight path. When the antennae are reattached, the moth regains its ability to control flight. These experiments show that antennae provide crucial mechanosensory cues for flight control. To investigate if the antennae play a similar role for flight stabilization also in bumblebees, similar experiments have recently been started with our Indian collaborators in our lab in Lund.

Bees with intact or manipulated antennae (sham, flagella cut off and whole antennae cut off (fig. 19)) are filmed flying down an experimental tunnel displaying strong visual cues. Our preliminary results from these ongoing experiments point towards some interesting differences between the flight speed and the lateral positioning of the untreated and antennectomized bees (the height above ground remains to be analyzed). The treated bees fly slower and with a larger lateral movement to their flight path than the untreated bees. This suggests that the antennae are important for flight stabilization, not only in butterflies and moths, but also in bumblebees. So far, we only have a limited data-set from 3 different bees from each treatment, but a growing set of data is currently being collected.
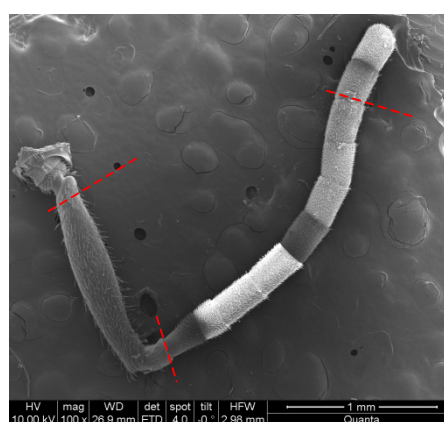
Fig. 19: Scanning electron micrograph of a bee antenna showing where the antennae are cut off for the three treatments (sham, flagella cut off and whole antennae cut off)

III.2. Inter-antennal angles of freely flying bees

The antennae of Lepidopterans have been hypothesized to act as organs of equilibrium, analogous to the halteres of dipterans. The Johnston's organs, which detect small-amplitude vibrations of the antenna during flight, are thought to facilitate this function. In the system of tethered bees, there is some evidence that antennae may be involved in sensing air-flow. Studies have shown that bees regulate their ground speed and compensate for headwinds using visual cues. Because there is also evidence that optic flow is involved in sensing ambient air flow via the mechanism of optomotor anemotaxis, it is important to understand how these results extend to the case of freely flying bees for which both visual and anemotactic feedback are relevant. As a first step, our collaborators from the National Centre for Biological Sciences (Bangalore, India), Taruni Roy and Dr. Sanjay Sane, have measured inter-antennal angles of freely flying bumblebees in response to varying ground speeds.

We have previously shown that bumblebees use axial optic flow to regulate their groundspeed. Bumblebees fly significantly faster in a tunnel lined with axial striped patterns on the wall compared to a tunnel lined with random chequerboard patterns.

Thus, altering visual patterns allows for experimental control of the bees' airspeed. Using this method, it is possible to plot inter-antennal angles as a function of airspeed (the speed of the bee with respect to the surrounding air mass) for bumblbees flying in approximately straight, forward trajectories. The results reveal a significant negative correlation between airspeed and inter antennal angle. Because the antennae are brought forward with increasing air speed against the force of drag due to head winds on the antennae, the antennae appear to respond actively to airflow. The function of this curious antennal response remains to be investigated

IV. ROLE OF COMBINED VISUAL AND MECHANOSENSORY INPUT IN FLIGHT CONTROL

IV.1. Role of vision and mechanosensation for landing

Insects are known to depend on visual cues to a large extent to initiate and execute a landing manoeuvre. Studies have used rotating spirals or moving gratings to simulate visual expansion to manipulate landing behavior. Recent work on landing behavior in honeybees suggest that they use a combination of visual and mechanosensory cues to guide and initiate the touchdown process.

During a set of preliminary experiments, we observed that bumblebees bring their antennae close to each other just before they land on a looming stimulus. To test this, we conducted a set of experiments in which bees were trained to land at the center of a rotating spiral. The spiral was connected to a DC motor in a tunnel of the same specifications and in the same filming conditions as the antennal positioning flight experiments. The walls of the tunnel were lined with a chequerboard pattern. Three experimental conditions were tested: 1) static spiral providing a simple looming stimulus for the bee, 2) contracting spiral (spiral rotated at 1.5 revolutions per second such that it generated contracting visual motion), 3) expanding spiral (spiral rotated at 1.5 revolutions per second such that it generated expanding visual motion). The results from this experiment are currently being analysed.

V. GENERAL CONCLUSION

As a result of using a wide variety of model organisms and approaches, we have made significant progress in this three-year period of the AFOSR contract in our investigations of the role of vision and mechanosensation in insect flight control. Some projects are still in progress or are nearing completion, whereas other have been completed and published in international journals. During the funding period we have accumulated a large amount of behavioral data, that has so far led to 2 published and one submitted paper (attached at the end of this report) and several others are in preparation. Our work has led to considerable understanding of how diurnal and nocturnal insects control flight visually, as well as by mechansosensation. These advances have all been detailed in this report. We are deeply grateful for the support we have received from the AFOSR.

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Minimum viewing angle for visually guided ground speed control in bumblebees

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SUMMARY

To control flight, flying insects extract information from the pattern of visual motion generated during flight, known as optic flow. To regulate their ground speed, insects such as honeybees and *Drosophila* hold the rate of optic flow in the axial direction (front-to-back) constant. A consequence of this strategy is that its performance varies with the minimum viewing angle (the deviation from the frontal direction of the longitudinal axis of the insect) at which changes in axial optic flow are detected. The greater this angle, the later changes in the rate of optic flow, caused by changes in the density of the environment, will be detected. The aim of the present study is to examine the mechanisms of ground speed control in bumblebees and to identify the extent of the visual range over which optic flow for ground speed control is measured. Bumblebees were trained to fly through an experimental tunnel consisting of parallel vertical walls. Flights were recorded when (1) the distance between the tunnel walls was either 15 or 30 cm, (2) the visual texture on the tunnel walls provided either strong or weak optic flow cues and (3) the distance between the walls changed abruptly halfway along the tunnel's length. The results reveal that bumblebees regulate ground speed using optic flow cues and that changes in the rate of optic flow are detected at a minimum viewing angle of 23–30 deg., with a visual field that extends to approximately 155 deg. By measuring optic flow over a visual field that has a low minimum viewing angle, bumblebees are able to detect and respond to changes in the proximity of the environment well before they are encountered.

Key words: bumblebee, flight control, flight speed, ground speed, vision.

INTRODUCTION

For safe and reliable navigation, flying insects require information about their current position, speed and orientation in space and information about the proximity of surfaces in their environment. This information is extracted, to a large extent, from the pattern of visual motion that is generated on the retina during flight. This pattern of apparent motion is called optic flow and comprises two components; rotational optic flow – caused by rotations *about* the roll, pitch or yaw axes – and translational optic flow, generated by translations *along* the roll, pitch or yaw axes (Koenderink, 1986). Unlike rotational optic flow, the translational optic flow varies with the distance to surfaces and the translational speed of the viewer with respect to these surfaces, i.e. surfaces that are closer generate higher image angular velocities than those that are further away. Properties of translational optic flow, such as the direction and velocity of motion in the visual scene, can thus provide cues for detecting the proximity of objects in the environment as well as information about the translational movement of the viewer (Gibson, 1950; Koenderink and van Doorn, 1987; Whiteside and Samuel, 1970).

Several investigations have revealed that translational optic flow cues are important for ground speed control in insects. In this context, ground speed is defined as the forward speed of the insect with respect to the ground, in contrast to its speed with respect to the air (airspeed). Honeybees (Baird et al., 2005; Srinivasan et al., 1996) and *Drosophila* (David, 1982; Fry et al., 2009) regulate their ground speed by holding constant the rate of translational optic flow in the axial, or front-to-back, direction (this type of optic flow will be referred to as axial optic flow). This strategy will ensure that ground speed is high when flying in an open field, where distances to surfaces are large, and low during flight through dense vegetation, where distances to surfaces are small and the chance of collision

high. Maintaining a constant image velocity on the retina may therefore function as a useful mechanism for ensuring that the speed of flight is automatically adjusted to a level that is safe and appropriate to the environment.

One interesting consequence of this strategy of ground speed control is that its performance varies with the angle at which axial optic flow is measured. When an insect flies from an open field into dense foliage, the apparent rate of axial optic flow will increase, causing the insect to decrease its ground speed. However, the point at which this change in proximity is first detected will vary with the minimum viewing angle (defined here as the angular deviation from the frontal direction of the longitudinal axis of the insect) at which changes in axial optic flow are perceived (Fig. 1). The lower the minimum viewing angle, the earlier the change in the surroundings that lie ahead will be detected. Early detection of a change in environment is advantageous because it would allow an insect to adjust its ground speed well before entering a new environment. This would be especially important when approaching dense, cluttered environments where a decrease in flight speed would increase the chances of detecting and avoiding nearby obstacles. One disadvantage of detecting changes in optic flow at a low viewing angle however, is that the magnitude of optic flow decreases non-linearly with decreasing viewing angle (Gibson, 1950), making the absolute difference in optic flow generated by a change in the environment more difficult to perceive. Furthermore, the signal will be more vulnerable to noise and detection errors. Increasing the minimum viewing angle at which changes in optic flow are detected would limit these errors, but would decrease the time between detecting a change in environment and entering the new environment – potentially increasing the chance of collisions. Despite the influence of viewing angle on the function of a visually guided ground speed

control strategy, very little is known about where in the visual field flying insects first start to measure optic flow for ground speed control.

One of the few behavioural experiments to investigate the visual angle at which optic flow for flight control is being measured was performed by Srinivasan et al. (Srinivasan et al., 1991). The aim of the study was to identify the region of the eye that was involved in the centring response – a behaviour in which honeybees balance the optic flow in each eye in order to fly between nearby obstacles. Honeybees appeared to respond to the presence of a black bar in an otherwise white experimental tunnel only when the bar passed the lateral region of the eye, indicating that the minimum viewing angle at which honeybees detect and respond to changes in optic flow lies in the lateral region of the visual field. This result is also consistent with the findings of an earlier study, which showed that honeybees use the image motion from landmarks in the lateral visual field to locate a frontally positioned target (Lehrer, 1990). These results indicate that, in honeybees, the lateral region of the visual field plays an important role in mediating optic-flow-driven tasks such as centring and short-range goal localisation.

The importance of viewing angle on the function of optic-flow-based behaviours has recently been demonstrated for visually guided unmanned aerial vehicles (UAVs). Hrabar et al. (Hrabar et al., 2006) showed that, in an environment consisting of parallel walls, the stability of the centring response in a UAV depends upon the angle at which optic flow is measured. In another study, Beyeler et al. (Beyeler et al., 2009) showed that the performance (measured as the flight duration before crashing) of a UAV using optic flow to avoid obstacles also changes significantly with the angle at which optic flow is measured. Both of these studies concluded that optimal performance was achieved when optic flow was measured at a single viewing angle of 45 deg. Interestingly, these empirical results from UAVs are in contrast to the more lateral viewing measured from the centring response in a flying insect (Srinivasan et al., 1991).

Riley et al. (Riley et al., 1999) hypothesised that bumblebees regulate their ground speed by holding constant the rate of axial optic flow from the ground beneath them. When flying in windy conditions, bumblebees do not maintain a constant ground speed. Instead, they tend to fly slower and lower in head winds, and faster and higher in tail winds. Based on these observations, the authors hypothesised that, to maintain constant the rate of optic flow beneath them when flying in strong head winds, bumblebees would have to decrease their height above the ground until the apparent rate of optic flow reaches the desired set point of the visual system. Similarly, in strong tail winds bumblebees would experience an increased rate of optic flow from the ground and would therefore increase their height to decrease the perceived rate of optic flow. However, Riley et al. (Riley et al., 1999) were unable to test their hypothesis as the harmonic radar information they used did not provide accurate data about the height at which the observed bumblebees were flying.

We have explored the properties of visual ground speed control in the bumblebee. We began by investigating the importance of visual cues for ground speed control and tested the hypothesis that, like honeybees and *Drosophila*, bumblebees regulate their ground speed by holding the rate of axial optic flow constant. We then examined further the mechanisms of visually guided ground speed control by investigating the response to an abrupt change in optic flow. In particular, our aim was to identify the minimum visual angle and the extent of the visual field over which bumblebees measure optic flow for ground speed control.

MATERIALS AND METHODS
Experimental animals

Bumblebees (*Bombus terrestris* L.) from commercial bumblebee hives (Koppert, UK) were used in the experiments. Each hive, containing approximately 200 individuals, was placed in an aluminium netting cage (2.3 m long, 2 m high and 2 m wide) at least 4 days before the experiments commenced. Several different hives were used over the course of the experiments. The cage was situated inside a room with two large windows that provided natural sunlight. The temperature within the room remained relatively stable during the experimental period, with experiments being performed at temperatures between 19 and 26°C.

In the initial stage of training, a plastic feeder containing sugar solution was placed near the hive entrance. Once a number of bees were regularly visiting the feeder, it was moved gradually into the experimental tunnel. In this way, a number of bees learned to visit the feeder at the end of the tunnel. These trained bees were then colour-marked using acrylic water-soluble paint for identification of individuals in the experiments.

Flight tunnel

The tunnels used in the experiments consisted of two parallel 30 cm high vertical walls and a smooth flat floor; both the walls and floor were 2 m in length [this method has been modified from that of Srinivasan et al. (Srinivasan et al., 1991)]. The top of the tunnel was covered with insect netting. Four DC light sources were placed around the tunnel to provide additional light for the experiments.

Experiment 1: measurement of the effect of optic flow on ground speed

The flights of bees flying to the feeder were recorded when the distance between the tunnel walls was set at a constant width of either 15 or 30 cm. In each case, the tunnel walls and floor were lined with a randomised chequerboard pattern consisting of 1 cm×1 cm black and white squares (Fig. 2A). The apparent rate of optic flow varies inversely with the distance to the visual environment. Thus, for a given ground speed, the apparent rate of optic flow perceived by a bumblebee flying in the centre of the tunnel will decrease as the distance between the tunnel walls increases.

To test the effect of visual cues (rather than other cues generated by changing the distance between the tunnel walls) on the regulation of ground speed, we minimised the axial optic flow cues in the tunnel by lining the tunnel walls and floor with an axial stripe pattern. This pattern consisted of alternating black and white, 3 cm wide stripes running the length of the tunnel (Fig. 2B). Although the axial optic flow cues generated by the random chequerboard pattern would be strong, flight in the direction of the stripes (along the long axis of the tunnel) would produce very little apparent axial optic flow on the retina. In this experiment, we tested the effect of minimising axial optic flow cues when the tunnel walls were either 15 or 30 cm apart.

Experiment 2: measurement of the effect of abrupt changes in tunnel width on ground speed

The distance between the walls was changed abruptly halfway along the tunnel, such that the distance between the walls in the first 1 m section of the tunnel was either larger or smaller than the distance between the walls in the second 1 m section. Flights of bees were recorded in two different tunnel configurations: 15 to 30 cm (Fig. 2C) and 30 to 15 cm (Fig. 2D). Two control experiments consisting of constant width 15 and 30 cm wide tunnels were also conducted and
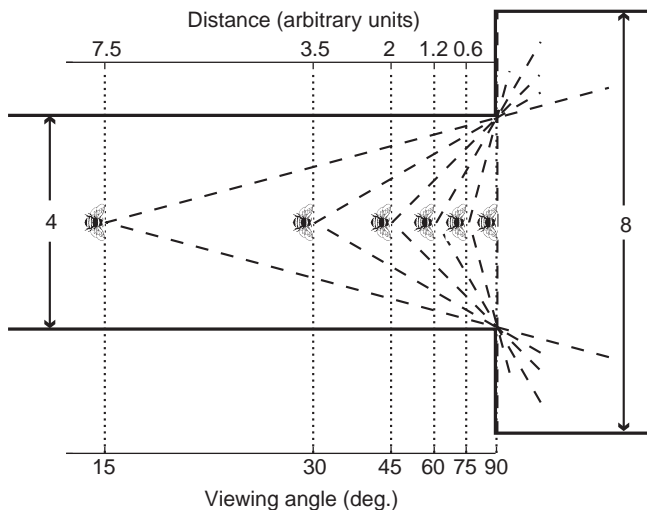
Fig. 1. Illustration of the relationship between the angle at which axial optic flow is measured and the distance at which a change in the proximity of the environment can be detected. Black lines represent the walls of an experimental tunnel. Dotted lines indicate the distance (top scale) at which the change in tunnel width first becomes apparent for each given viewing angle (defined as the angular deviation from the longitudinal body axis, bottom scale) for a bee flying along the midline of the tunnel.
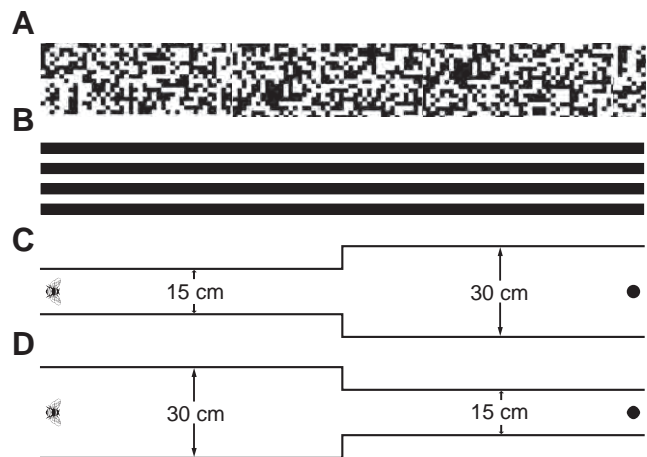

Fig. 2. Experimental patterns and setup. (A) The randomised chequerboard pattern used to generate strong axial optic flow cues and (B) the axial stripe pattern used to generate weak axial optic flow cues in experiment 1. (C) The 15 to 30 cm and (D) the 30 to 15 cm abrupt tunnel configurations used in experiment 2; black lines represent the tunnel walls. The feeder is indicated as a black circle.

the results were compared with those obtained in the variable width tunnels. In this experiment, the texture on the floor of the tunnels was removed in order to simplify our estimations of the visual region that was being used to measure optic flow for ground speed regulation. Thus, in all of these experimental conditions, the walls of the tunnel were lined with a randomised chequerboard pattern whereas the floor was blank white, providing minimal visual features. In a further experimental condition, flights of bees were recorded when the distance between the walls remained constant at 15 cm but the pattern on the walls changed abruptly in the centre of the tunnel from the chequerboard to the axial stripe pattern.

Recording and analysis of flight trajectories

The bees were allowed to visit the feeder at the end of the experimental tunnel for at least 1 day before recording commenced. Trials for each experimental condition were conducted over 2–3 days. Flights of bees flying through the tunnel to the feeder were recorded at 60 Hz using a Mikrotron MotionBLITZ EoSens (Unterschleisheim, Germany) camera mounted above the centre of the tunnel. The position of the bee and the orientation of the long axis of the body were determined using an automated tracking program (Lindemann, 2005). The bee position data was calibrated using the output of the Camera Calibration Toolbox for Matlab (Bouguet, 1999) and converted to metres using known size reference patterns placed at different heights from the tunnel floor. The relationship between metre and pixel distances was such that it varied by less than 0.01 m per pixel between the floor and top of the tunnel. This meant that, by using an intermediate metre per pixel value, the distance error associated with bees flying at different heights in the tunnel would be minimised.

To avoid pseudo-replication, the data from repeated flights from individual bees were averaged so that each individual was considered only once per experimental treatment. Data from bees that flew a minimum of two times were included in the analysis. Non-parametric Wilcoxon rank sum tests at the 5% significance level were performed on the data.

Ground speed was calculated by finding the two-dimensional distance travelled between successive frames and dividing this value by the time step between the frames, 0.0167 s. Ground speed was calculated over a distance of 0.8 m in the central section of the tunnel. In experiment 2, ground speed data was averaged over 2 cm bins. The ground speed at each 2 cm step was then directly compared with the ground speed in the relevant control condition (15 cm constant width tunnel for the 15 to 30 cm abrupt change tunnel and 30 cm constant width tunnel for the 30 to 15 cm abrupt change tunnel – no texture on the floor) using Wilcoxon rank sum tests. A change in ground speed was deemed to occur when the difference between the ground speed in the abrupt tunnel and the ground speed in the control tunnel were significant (at the 5% level) and remained significant for the remaining distance of the tunnel. In the second half of the tunnel, ground speed was deemed to have reached the speed obtained in the control tunnel of the same width when there was no longer a significant difference between these values.

RESULTS
Experiment 1: effect of axial optic flow on ground speed control

In this experiment, we compared the ground speeds of bees flying in the experimental tunnel when the distance between the walls was either 15 or 30 cm. We recorded 34 flights from seven bees in the 15 cm tunnel and 40 flights from six bees in the 30 cm tunnel. Bumblebees fly significantly faster in the 30 cm tunnel, $0.46 \pm 0.09 \, \text{m s}^{-1}$ (mean ± s.d.), than in the narrower 15 cm tunnel, $0.29 \pm 0.05 \, \text{m s}^{-1}$ (Wilcoxon rank sum, $N=13$, $P<0.001$; Fig. 3). One possible explanation for the difference in ground speed between the two tunnels is that the bees were changing their ground speed in response to the different mechanosensory cues present in each tunnel. To test this possibility, we recorded the flights of bees flying in both the 15 and 30 cm wide tunnels when the pattern on the walls and floor provided very weak axial (front-to-back) optic flow cues. We recorded 26 flights from nine bees in the 15 cm tunnel and 30 flights from eight bees in the 30 cm tunnel. Bumblebees flew at $0.98 \pm 0.23 \, \text{m s}^{-1}$ in the 15 cm tunnel, and at $0.92 \pm 0.28 \, \text{m s}^{-1}$ in the
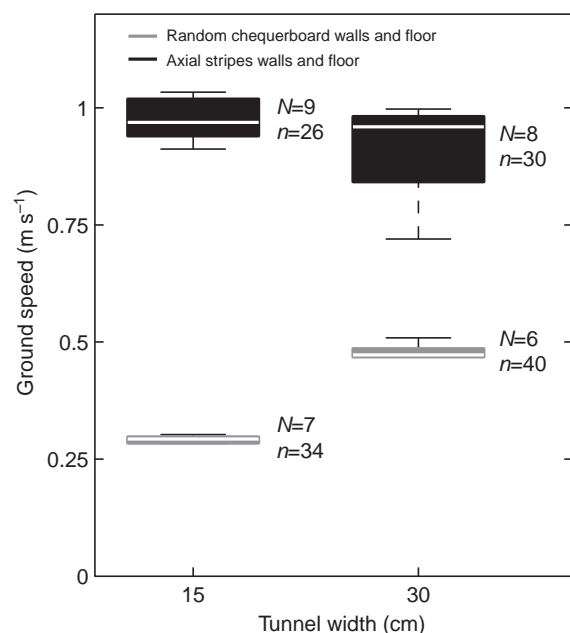
Fig. 3. Effect of axial optic flow on ground speed control. A box plot of the ground speed of bees flying in either a 15 or 30 cm wide tunnel when the walls and floor are lined with a random chequerboard pattern (grey boxes), or an axial stripe pattern (black boxes). Boxes indicate the distance between the lower and upper quartile values, white lines indicate the median values and whiskers indicate the entire spread of the data. *N* is the number of bees, *n* is the number of flights.
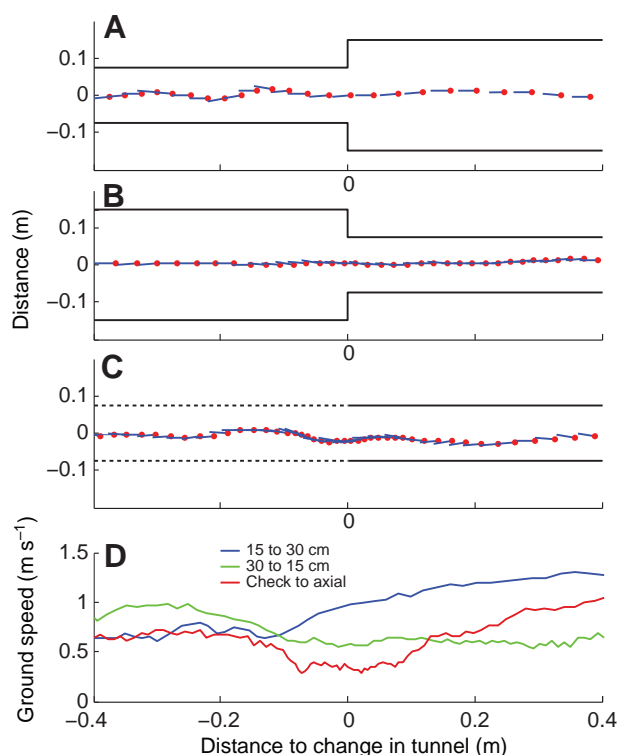

Fig. 4. Example of flight trajectories of bumblebees flying in the experimental tunnel when the width of the tunnel changed from 15 to 30 cm (A), 30 to 15 cm (B) or when the tunnel width remained constant but the pattern changed from chequerboard to axial stripes (C). Red circles represent the position of the bee every 0.033 s, blue lines indicate the orientation of the body long axis, black lines represent the tunnel walls. (D) The ground speed of the flight trajectories shown in A (blue line) B (green line) and C (red line).

30 cm tunnel (Fig. 3). When the tunnel was lined with axial stripes, there was no longer an effect of tunnel width on ground speed (Wilcoxon rank sum, $N=17$, $P=0.606$). The results of this experiment indicate that the bumblebees are relying primarily on axial optic flow cues to regulate ground speed. This theory is further supported by the two- to threefold increase in ground speed recorded in the axial stripe tunnels compared with that observed in the tunnels lined with chequerboard patterns. An increase in the ground speed is exactly what can be expected from a system that aims to hold constant the rate of translational optic flow between the two sets of tunnels.

Experiment 2: effect of abrupt changes in tunnel width on ground speed

In this experiment, we recorded the ground speed of bees flying in the experimental tunnel when the distance between the walls changed abruptly (from 15 to 30 cm or from 30 to 15 cm), 1 m along the length of the tunnel (example flight trajectories are shown in Fig. 4). These data were compared with data from two control conditions, a 15 cm wide constant width tunnel and a 30 cm wide constant width tunnel. We recorded 56 flights from nine bees in the 15 cm control condition, 45 flights from 11 bees in the 30 cm control condition, 57 flights from 19 bees in the 15 to 30 cm condition and 35 flights from eight bees in the 30 to 15 cm condition.

In the 15 to 30 cm tunnel, the ground speed of bees increased significantly from the ground speed in the 15 cm wide constant width tunnel at a distance of 0.14 m before the change in tunnel width (Wilcoxon rank sum, $N=28$, $P=0.001$; Fig. 5A). In the second half of the tunnel, ground speed reached the same level as in the 30 cm tunnel at a distance of 0.16 m after the change in tunnel width

(Wilcoxon rank sum, $N=28$, $P=0.16$; Fig. 5A). In the 30 to 15 cm tunnel, the ground speed of bees decreased significantly from the ground speed in the 30 cm wide constant width tunnel at a distance of 0.26 m before the change in tunnel width (Fig. 5B; Wilcoxon rank sum, $N=19$, $P=0.002$). In the second half of the tunnel, ground speed reached the same level as the ground speed in the 15 cm tunnel at a distance of 0.18 m after the change in tunnel width (Wilcoxon rank sum, $N=19$, $P=0.23$; Fig. 5B). The results of this experiment again reveal that the bees do indeed change their ground speed in response to the change in tunnel width. More interestingly, in both conditions, the ground speed of the bees changed significantly from the control condition well before the bees had reached the change in tunnel width. The results also indicate that bumblebees do not reach the expected ground speed for the new tunnel width until some distance after the change.

Thirty flights from 11 bees were recorded in a tunnel of constant width, but the pattern changed abruptly from a chequerboard pattern to an axial stripe pattern halfway along its length. This experiment was designed to control for the possibility that the bees change their flight speed primarily as a response to the physical change in the distance between the tunnel walls – rather than to the change in the rate of axial optic flow. Again, ground speed changed significantly from the control condition, at –0.18 m, well *before* the bee has reached the change in patterns (Fig. 5C; Wilcoxon rank sum, $N=20$, $P=0.01$). This result supports the conclusion that the visual field
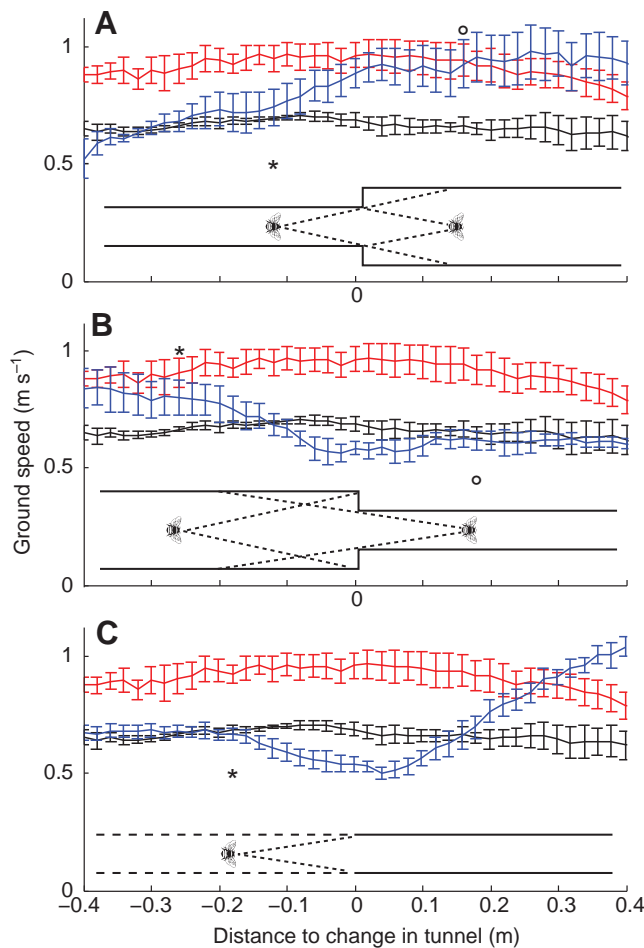
Fig. 5. Effect of abrupt changes in tunnel width on ground speed. Effect of abrupt changes in axial optic flow on ground speed (blue lines) when the width of the tunnel changes from 15 to 30 cm (A), from 30 to 15 cm (B) or when the pattern in a 15 cm wide constant width tunnel changes from random chequerboard to axial stripes (C). Black lines represent the mean ground speed of bumblebees flying in the 15 cm wide constant width tunnel, and red lines represent the mean ground speed of bumblebees flying in the 30 cm wide constant width tunnel. Means are calculated over 2 cm bins, error bars represent the standard deviation of data within each 2 cm bin (see Materials and methods). Asterisks indicate the position at which the test condition (blue lines) deviates significantly from the control condition (15 cm constant width for A and C; 30 cm constant width for B). Circles indicate the position at which the test condition first reaches the level of the control condition in the second half of the tunnel (30 cm constant width for A; 15 cm constant width for B). We recorded 45 flights from 11 bees in the 30 cm control condition, 56 flights from nine bees in the 15 cm control condition, 57 flights from 19 bees in the 15 to 30 cm condition (A), 35 flights from eight bees in the 30 to 15 cm condition (B) and 30 flights from 11 bees in the chequerboard to axial condition (C).

over which bumblebees are measuring axial optic flow cues to regulate ground speed begins at a relatively low visual angle.

Based on our observations from experiment 1, we would expect the bees to speed up when they encounter the axial stripe pattern. However, the bees responded to the change in patterns by decreasing their ground speed and did not speed up until they had passed the change. This drop in speed is most likely a response to the sudden disappearance of axial optic flow cues. Once the bees had flown past this change, they did, however, increase their ground speed to

a value that greatly exceeded the ground speed recorded in the first section of the tunnel.

Observations of flight trajectories

To be able to approximate the extent of the visual field over which bumblebees detect and respond to changes in optic flow, it is necessary to obtain information about the lateral position and orientation of the bees as they fly through the tunnel. The lateral distance from the midline of the tunnel of bees flying in the 15 to 30 cm tunnel is 0.01±0.005 m (mean ± s.d.) before the change in tunnel width. In the 30 to 15 cm tunnel, the mean lateral position before the change in tunnel width is 0.01±0.008 m. The orientation of the head, and therefore the visual field, is also crucial for understanding what visual information the bees receive as they approach the change in tunnel width. From the recordings taken in this experiment, it was not possible to resolve the head position. However, subsequent observations of close-up images taken of bumblebees flying in the tunnel reveal that the head is oriented in line with the long axis of the body for most of the time (data not shown). The orientation of the long axis of the body (which could be resolved in the recordings taken during this experiment) therefore appears to provide a reasonable indication of the orientation of the visual field. The mean body orientation (with zero representing orientation along the long axis of the tunnel in the direction of the feeder) is 2±9 deg. before the change in tunnel width in the 15 to 30 cm tunnel, and 4±9 deg. in the 30 to 15 cm tunnel. These results indicate that the position of the bumblebees as they approach the change in tunnel width can be approximated as being centred along the midline of the tunnel and oriented along its long axis.

DISCUSSION
The role of axial optic flow in ground speed control

The results presented above show that bumblebees rely primarily on visual cues to regulate ground speed. In the 30 cm wide tunnel, bumblebees fly significantly faster than in a tunnel that is half as wide (Fig. 3). The apparent rate of axial optic flow experienced by bumblebees flying in the tunnel is inversely proportional to the distance between the tunnel walls. As the distance between the walls increases, the apparent rate of axial optic flow decreases and, as a result, bumblebees increase their ground speed. The importance of axial optic flow cues for ground speed control in bumblebees is highlighted by the result that, when the axial optic flow cues are removed (such as when the pattern in the tunnel is composed of axial stripes), bumblebees fly significantly faster than when these cues are present (random chequerboard pattern; Fig. 3). Moreover, in the presence of axial stripes, the relationship between ground speed and tunnel width disappears. Instead, bumblebees now fly at a constant speed, even when the distance between the tunnel walls is doubled (Fig. 3).

Drosophila (David, 1982; Fry et al., 2009) and honeybees (Baird et al., 2005; Srinivasan et al., 1996) regulate ground speed by holding constant the rate of axial optic flow. A consequence of this strategy is that ground speed will increase proportionally with the distance to nearby surfaces. If bumblebees also control their ground speed in this way, we expect a factor of two increase in the width of the experimental tunnel to result in a factor of two increase in ground speed. However, in our experiments, ground speed increased by only a factor of 1.6 between the 15 cm and the 30 cm tunnels, resulting in only a partial compensation for the change in tunnel width. Nonetheless, this partial compensation was robust across many individuals and across different hives, suggesting that it is truly a consequence of the bumblebee's ground speed control strategy and

that this strategy differs somewhat from that which is observed in *Drosophila* and honeybees. Additional information about ground speed could be derived from either visual or mechanosensory cues, or a combination of both (see below). A weighed sum of these cues and axial optic flow cues could then underlie the partial compensation in ground speed to the two-fold change in tunnel width.

Bumblebees also seem to use a different strategy to honeybees when tested in an environment with only very weak optic flow cues. Barron and Srinivasan (Barron and Srinivasan, 2006) showed that, when flying in an experimental tunnel lined with axial stripes, honeybees fly faster in a wide tunnel than they do in a narrower one. By contrast, bumblebees fly at a constant speed, irrespective of the width of the tunnel (Fig. 3). Whether this difference between honeybees and bumblebees is due to a difference in the sensitivity of the motion detection mechanisms of these insects, a fundamental difference in the ground speed control strategies or some other difference, remains to be tested.

What is the visual range over which axial optic flow for ground speed control is being measured?

Bumblebees begin to adjust their ground speed in response to an abrupt change in axial optic flow cues some distance *before* they pass the point at which these changes occur. This is clear from all of our experimental conditions that present the bumblebees with an abrupt change in the rate of axial optic flow (Experiment 2). If the lateral position and orientation of the bee with respect to the walls of the tunnel are known, it is possible to calculate the visual angle occupied by the change in tunnel width at the position where the change in ground speed occurs (Fig. 6A).

Our data indicate that the mean position and orientation of bumblebees is centred along the midline of the tunnel and oriented toward the feeder. Thus, according to our calculations, the change in ground speed takes place when the change in tunnel width occupies a visual angle of approximately 28 deg. in the 15 to 30 cm tunnel and 30 deg. in the 30 to 15 cm tunnel. Interestingly, when the tunnel walls remained at a constant width but the pattern changed from chequerboard to axial stripes, the change in ground speed took place when the change in pattern occupied a smaller visual angle of approximately 23 deg. This indicates that bumblebees are able to respond to changes in the rate of axial optic flow within a visual field whose minimum angle lies approximately between 23 and 30 deg. from the frontal direction of the midline. It is important to note that the calculation of the viewing angle at which bees first respond to changes in optic flow is derived from the point at which a change in flight speed occurs. Thus, these values do not take into account the processing delay between the detection of a change in optic flow and the change in ground speed. This processing delay has been estimated at 100 ms for *Drosophila* (Fry et al., 2009). If we use this as an approximate value for the processing delay in bumblebees, we estimate that the minimum viewing angle at which bumblebees first detect a change in optic flow is approximately 20 deg. in the 15 to 30 cm tunnel, 24 deg. in the 30 to 15 cm tunnel and 17 deg. in the axial stripe tunnel.

One obvious advantage of detecting changes in optic flow at low viewing angles is that changes in the density of the environment would be detected well before the bee enters the new surroundings. This would give the visual system time to detect the change in axial optic flow and the motor system time to adjust ground speed appropriately. By contrast, if changes in axial optic flow are first being detected at more lateral viewing angles, new environments may be encountered before the visual and flight motor systems have had time to detect and respond to it. Interestingly, both the centring
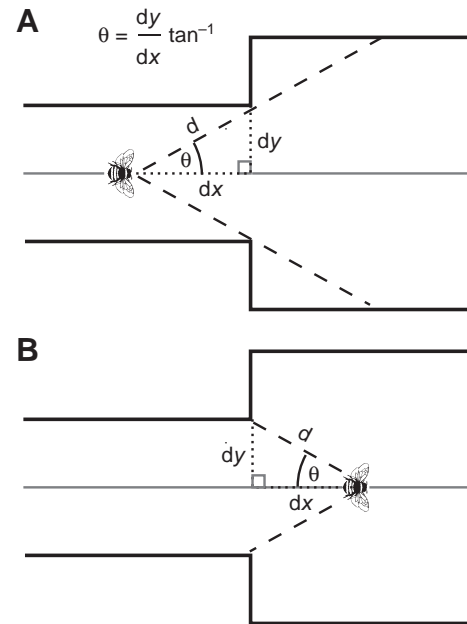

Fig. 6. Calculation of the angular range over which bumblebees measure axial optic flow for flight speed control. Illustration of the calculation of the viewing angle θ occupied by the change in tunnel width when ground speed initially changes in response to a change in tunnel width (A) or when reaches the same value as the control in the second half of the tunnel (B). Note that, in B, θ is subtracted from 180 deg. to obtain the maximum angle at which optic flow for ground speed control is being measured, with respect to the frontal direction of the midline of the bee. Black lines indicate the tunnel walls; the grey line indicates the midline of the tunnel.

response (Srinivasan et al., 1991) and short-range goal localisation (Lehrer, 1990) in honeybees are mediated by optic flow cues in the lateral visual field. Further investigations into the minimum viewing angles that different insects use for different behaviours are necessary to understand the full extent of the role that viewing angle has on the many components of flight control.

It is possible to estimate the extent of the visual region over which optic flow for ground speed control is being measured by calculating the distance at which ground speed in the second half of the tunnel reaches the same value as the equivalent constant-width tunnel (Fig. 6B). In the 15 to 30 cm tunnel and the 30 to 15 cm tunnels, ground speed reaches its new value when the first half of the tunnel subtends a visual angle of 155 deg. and 140 deg., respectively. If we again factor in a processing delay of 100 ms, these viewing angles are reduced to 132 deg. and 129 deg.. Our calculations thus suggest that the visual field over which bumblebees are measuring optic flow for ground speed control extends between approximately 17 deg. to 132 deg. It is important to note, however, that the rate at which a bumblebee can speed up or slow down in response to changes in optic flow is not known. As such, the maximal viewing angles calculated here are only approximate indicators of the true extent of the visual field that is used to measure optic flow for ground speed control. Nonetheless, our results do suggest that optic flow for ground speed control is being measured over an extensive visual range.

How do bumblebees detect changes in optic flow?

There are two distinct hypotheses to explain how bumblebees use optic flow for ground speed control. In the first hypotheses, visual information across the entire visual region is assigned equal weights,
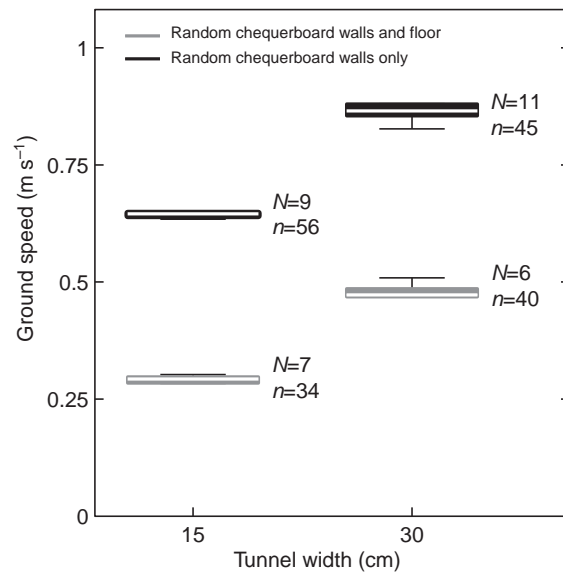
Fig. 7. Effect of ventral optic flow cues on ground speed in tunnels of different widths. The ground speed of bumblebees flying in either a 15 or 30 cm wide tunnel when either the walls and floor (grey boxes) or walls only (black boxes) are lined with a random chequerboard pattern. Details as in Fig. 3.

such that each area of the visual field has equal influence over the overall optic flow measurement. According to this model, a change in ground speed would be initiated only when a change in optic flow subtends a large enough area of the visual field to influence the total output of motion-sensitive neurons. This model would predict that bumblebees respond equally to changes in optic flow, irrespective of where in the visual region they occur, provided that they are large enough to influence the overall output. In the second hypothesis, information from neurons across the visual field is weighted unevenly, such that information from a particular area has more influence over the total optic flow output. If, for example, neurons in the forward-looking sector of the visual region have a higher weighting, then changes in the upcoming visual scene will have a stronger influence over the overall optic flow output than changes that occur at larger visual angles. The plausibility of this hypothesis is supported by the discovery that wide-field-motion-sensitive neurons in the visual system of the fly exhibit increased sensitivity to motion in the frontal part of the visual field (Hausen, 1982). It is not possible from the present study to determine which model best describes visual processing in bumblebees. Further experiments investigating how bumblebees respond to changes in optic flow that occur in different parts of the visual field are necessary to better understand the mechanisms that underlie ground speed control.

The role of ventral optic flow in ground speed control
It is probable that the regions of the visual field that are used for measuring optic flow for ground speed control have a ventrally oriented component, particularly because they would provide useful information about ground speed in an open environment where lateral optic flow cues are sparse. Evidence for the importance of ventral optic flow cues for ground speed control is provided by the result that bumblebees fly at different ground speeds when optic flow in the ventral visual field is present or absent. When optic flow cues on the floor of a constant width tunnel are removed (experiment

1 and control conditions for experiment 2), bumblebees fly faster than when these cues are present (Fig. 7). The effect of removing ventral optic flow cues on ground speed control is consistent with the results from similar experiments in honeybees (Baird et al., 2006), highlighting the importance of these cues in ground speed control in these and possibly other flying insects.

Secondary cues for ground speed control
The elevated ground speed that was observed in the axial stripe tunnels (~0.9 m s^{-1}) is significantly slower than the speed at which bumblebees are capable of flying in an open environment (~7 m s^{-1}) (Riley et al., 1999). Thus, even in the absence of strong axial optic flow cues, bumblebees appear to be able to extract some information about the proximity of the environment and their ground speed within it, causing them to reduce their speed to a relatively low value. This is consistent with the results of Baird et al. (Baird et al., 2005) and Barron and Srinivasan (Barron and Srinivasan, 2006), which showed that, although honeybees fly nearly three times faster in a tunnel lined with axial stripes relative to one lined with a chequerboard pattern, this speed is still much slower than the speed at which honeybees are capable of flying outdoors. The downregulation of ground speed inside a narrow tunnel as compared with a flight outdoors suggests that bumblebees are able to obtain ground speed information from cues other than the axial optic flow cues present within the tunnel.

Examples of visual cues that could provide ground speed information are axial optic flow cues generated by structures above the experimental arena (such as the camera and light fittings), or expansion cues produced by flight at angles that are oblique to the longitudinal axis of the tunnel (in the case of the axial stripe tunnel, these would be limited to vertical expansion cues). Mechanosensory cues, such as airspeed information, could also provide the bees with important information about how fast they are flying relative to the air. In the relatively still air of the experimental tunnel, airspeed would provide a direct indication of ground speed. Evidence that bumblebees do not rely primarily on airspeed cues for ground speed control comes from the results of experiment 1, which showed that ground speed is significantly faster when the tunnel is lined with axial stripes than when it is lined with a chequerboard pattern. This makes sense because dependence on airspeed cues for determining ground speed would provide unreliable information in the natural habitat of the bumblebee, where the airflow patterns are turbulent and unpredictable. Future investigations are required to determine what additional visual and/or mechanosensory cues are involved in bumblebee ground speed control.

Conclusions
The results of the present study reveal that bumblebees regulate their ground speed using axial optic flow cues. Our investigation also shows that, although the rate axial optic flow is the primary cue used by bumblebees for ground speed control, information from other visual and/or mechanosensory sources is also used to mediate this behaviour. Nonetheless, our results indicate that changes in the rate of axial optic flow in the frontal visual field are sufficient to generate a change in ground speed. This strategy of regulating ground speed using optic flow cues from the frontal visual field enables bumblebees to detect and respond to changes in the density of the environment before the new environment is entered. This would be of particular importance when flying from a wide-open field into the cluttered environment around bushes and trees, where it is important to reduce ground speed in order to avoid obstacles. Our study also suggests that optic flow for ground speed control is

being measured over a broad visual field, extending well beyond the lateral view of the insect.

The mechanisms of ground speed control investigated in this study focus on the effect of symmetrical changes in optic flow in a relatively cluttered visual environment. What remains unclear is how less cluttered and more natural environments influence ground speed control. Is the relationship between flight speed and proximity constant, or is there a maximum distance beyond which changes in ground speed are no longer observed? Of equal importance is understanding the effect of asymmetrical changes – such as those that may occur when an insect flies beside a hedge or a forest – on ground speed control in flying insects. Our results also raise interesting questions about the role of viewing angle in other visually guided flight control behaviours, in bumblebees as well as in other insects. Does the minimum viewing angle identified here represent the minimum point of the visual range over which other flight control behaviours are mediated, or are different visual regions specialised for mediating different flight control behaviours? Future investigations will be focussed on addressing these questions in both bumblebees and other flying insects.

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Nocturnal insects use optic flow for flight control

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To avoid collisions when navigating through cluttered environments, flying insects must control their flight so that their sensory systems have time to detect obstacles and avoid them. To do this, day-active insects rely primarily on the pattern of apparent motion generated on the retina during flight (optic flow). However, many flying insects are active at night, when obtaining reliable visual information for flight control presents much more of a challenge. To assess whether nocturnal flying insects also rely on optic flow cues to control flight in dim light, we recorded flights of the nocturnal neotropical sweat bee, *Megalopta genalis*, flying along an experimental tunnel when: (i) the visual texture on each wall generated strong horizontal (front-to-back) optic flow cues, (ii) the texture on only one wall generated these cues, and (iii) horizontal optic flow cues were removed from both walls. We find that *Megalopta* increase their groundspeed when horizontal motion cues in the tunnel are reduced (conditions (ii) and (iii)). However, differences in the amount of horizontal optic flow on each wall of the tunnel (condition (ii)) do not affect the centred position of the bee within the flight tunnel. To better understand the behavioural response of *Megalopta*, we repeated the experiments on day-active bumble-bees (*Bombus terrestris*). Overall, our findings demonstrate that despite the limitations imposed by dim light, *Megalopta*—like their day-active relatives—rely heavily on vision to control flight, but that they use visual cues in a different manner from diurnal insects.

Keywords: flight; optic flow; insect vision; *Megalopta*; bumble-bee

1. INTRODUCTION

Nocturnal sweat bees, *Megalopta genalis* (Halictidae), live in hollowed-out sticks in the tangled understories of neotropical rainforests and are active in the dim light conditions that occur just before sunrise and after sunset [1,2]. To forage, these bees must negotiate the dark and cluttered environment around their nests, fly to a flowering tree to collect nectar and pollen and then find their way back home again [3]. Like all hymenopterans, *Megalopta* possess apposition compound eyes, which are adapted for vision in bright light. Although *Megalopta* have evolved optical specializations to capture more light than their day-active relatives, these enhancements are not sufficient to

explain how *Megalopta* capture enough light to use vision in their dim habitat [4]. Despite this limitation, *Megalopta* use visual landmark information to locate their nests at very low light intensities [4].

Day-active insects such as honeybees [5,6], bumble-bees [7,8] and flies [9,10] use information extracted from the pattern of visual motion that occurs on the retina during flight (known as optic flow) to control groundspeed. A consequence of this strategy is that speed increases dramatically when horizontal optic flow cues are minimized [5,11]. Another behaviour known to be mediated by horizontal optic flow is the 'centring' response. When flying through narrow gaps, the day-active honeybee 'centres' between the nearby surfaces by balancing the rate of horizontal optic flow experienced in each eye [12]. This strategy ensures that the insect will maintain an equal distance between obstacles without the need for absolute distance measurements. When the rate of horizontal optic flow experienced on each eye becomes imbalanced, the insect attempts to restore the balance by flying nearer to the surface that provides the least horizontal optic flow.

As light intensity decreases, however, the perception of the pattern of visual motion is corrupted by noise and becomes decreasingly reliable. Do insects that are active in dim light also rely on optic flow to control flight, despite the reduced reliability of visual information? Here, we explore the limits of dim light vision by investigating whether *Megalopta* flying at low light intensities are also able to use optic flow cues for groundspeed control and centring. We also compare the visual flight-control strategies of *Megalopta* and a diurnal bee, *Bombus terrestris* (Apidae).

2. MATERIAL AND METHODS

Nest sticks of *M. genalis* were collected on Barro Colorado Island in Panama and transferred to the experimental site (see [1] for site description). To explore the differences between the visual flight-control strategy of *Megalopta* and a day-active hymenopteran, we repeated the experiment using *B. terrestris* from a commercial hive (Koppert, UK) located outdoors near Lund, Sweden.

The experimental set-up consisted of a Perspex tunnel, 14 cm wide × 14.5 cm high × 50 cm long, mounted 65 cm above the ground. The nest/hive was placed at an opening in one end of the tunnel such that, to exit or enter their nest/hive, the bees had to fly along the tunnel's length. The walls of the tunnel were lined with either a pattern consisting of randomly placed black-and-white 3 × 3 cm squares ('check'), or a pattern of alternating black-and-white 3 cm wide horizontal stripes ('stripe'). For a bee flying along the tunnel, the check pattern provided strong horizontal optic flow cues, whereas the stripe pattern provided minimal horizontal visual cues. The effect of horizontal optic flow cues on flight control was tested under three conditions: (i) check/check—both walls displayed the check pattern, (ii) check/stripe—one wall displayed the check pattern and one displayed the stripe pattern, and (iii) stripe/stripe—both walls displayed the stripe pattern. The three conditions were presented to the bees in a randomized order, with each condition being presented four times. The numbers of recorded flights were 24 and 28 (check/check), 21 and 32 (check/stripe), 24 and 31 (stripe/stripe), for *Megalopta* and *Bombus*, respectively.

Flights of bees returning to their nest were recorded (at 25 and 50 frames s^{-1}; *Megalopta* and bumble-bees, respectively) using a camera mounted underneath the tunnel. The top panel of the tunnel was sandblasted to make a light background against which the bees could be distinguished in the films. Recording sessions were performed during the normal foraging times (*Megalopta*: 45 min before sunrise and 45 min after sunset [1,2]; *Bombus*: two 30 min periods, at 10.30 and 13.30 h). Light intensity varied between 1–12 lux for *Megalopta* and 12 000–20 000 lux for *Bombus*.

Groundspeed was calculated by finding the longitudinal distance travelled between successive frames and dividing this value by the time step between the frames. This data was then averaged. Centring
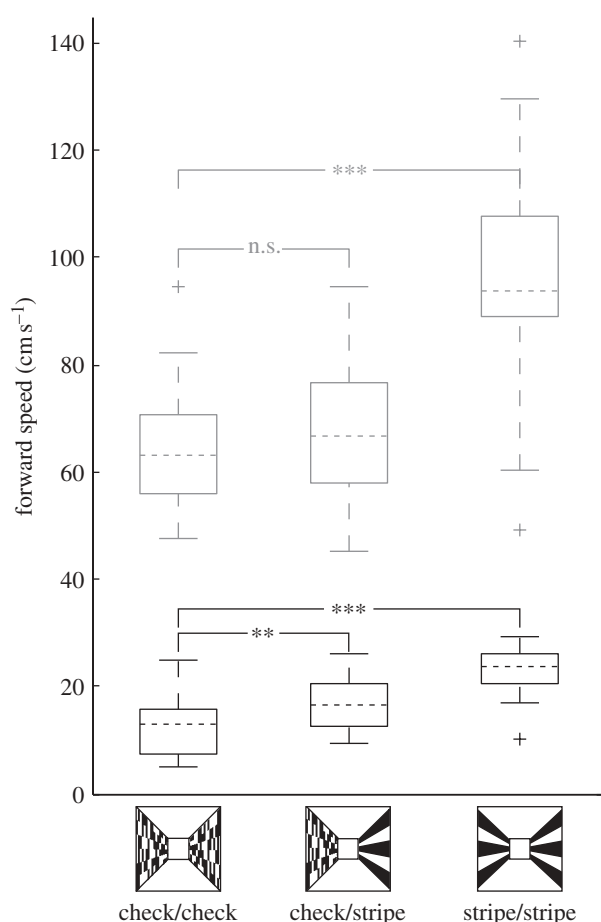
Figure 1. The effect of changes in horizontal optic flow cues in the tunnel on the groundspeed of *M. genalis* (black boxes) and *B. terrestris* (grey boxes). Box limits represent the 25th and 75th percentiles of the data, dotted lines indicate the median, whiskers extend to the rest of the data, crosses indicate outliers. Both species increase their groundspeed when horizontal motion cues are minimized, but unlike *Megalopta*, bumble-bees do not fly faster when horizontal motion cues are removed from one wall. Significance codes: **$p < 0.01$; ***$p < 0.001$; n.s., not significant.

was calculated by finding the average lateral distance from the midline of the tunnel. For analysis, we calculated the groundspeed and centring over the first 25 cm of the tunnel to avoid including landing manoeuvres at the nest/hive.

The effect of experimental condition on groundspeed and centring was assessed using analysis of variances (ANOVAs) and Student's *t*-tests at the 5 per cent significance level. Linear mixed model analyses [13] using the lme function in R (release 1.26) with light intensity as a random effect were used to account for the level of variation, which is introduced by recording flights at different light intensities.

3. RESULTS
When the patterns on the walls of the tunnel provided decreasing amounts of horizontal optic flow cues—check/stripe, and stripe/stripe condition in comparison with the check/check condition—groundspeed in *Megalopta* increased (figure 1; check/stripe condition: $t_{43} = 3.30$, $p = 0.002$; stripe/stripe condition: $t_{46} = 7.81$, $p < 0.0001$). However, the amount of horizontal optic flow present in the tunnel had no effect upon the average centring (lateral position) of the bees as they flew along the tunnel (figure 2a; one-way ANOVA: $F_{2/66} = 0.79$, $p = 0.46$).

Interestingly, in all conditions, the diurnal bumble-bees fly considerably faster than *Megalopta* (figure 1). Like *Megalopta*, bumble-bees increase their groundspeed when horizontal motion cues are minimized (figure 1; stripe/stripe compared with check/check condition: $t_{57} = 8.11$, $p < 0.0001$). Unlike *Megalopta*, however, bumble-bees do not fly faster when horizontal motion cues are removed from only one wall (check/stripe compared with check/check condition: $t_{58} = 0.78$, $p = 0.44$). Another difference between *Megalopta* and bumble-bees is the effect of horizontal motion cues on centring. Whereas an asymmetry in horizontal optic flow did not affect centring in *Megalopta*, it caused the bumble-bees to fly closer to the wall that displayed the stripe pattern in the check/stripe condition (figure 2b; comparison with check/check condition: $t_{58} = 6.68$, $p < 0.0001$). There was no difference in the average distance from the midline between the stripe/stripe and the check/check conditions in bumble-bees ($t_{57} = -0.75$, $p = 0.46$).

4. DISCUSSION
In this study, we compared the flight control of two different bee species adapted for flight at radically different light intensities. The most striking result of this comparison is the difference in the speed at which *Megalopta* and bumble-bees fly. When optic flow cues are strong, the mean groundspeed of bumble-bees in the tunnel is over five times faster than that of *Megalopta*. One way to improve visual reliability in dim light is to integrate the visual signal over time; a process called temporal summation. A consequence of temporal summation is the inability to detect high rates of optic flow, so the animal has to reduce its speed in order to perceive self-generated visual motion. The relatively low groundspeed of *Megalopta* lends support to the behavioural [14] and theoretical [15] indications that these bees use temporal summation to help them to perceive optic flow and to use it for flight control.

Further evidence for the importance of vision for flight control in *Megalopta* is provided by the finding that groundspeed increases when optic flow cues are minimized. This response is similar to the behaviour of honeybees [5,6] and bumble-bees [8,11], indicating that optic flow information is used in a similar manner for groundspeed control. However, other observed differences between the flight-control behaviours of *Megalopta* and bumble-bees suggest that *Megalopta* is using optic flow information in a different way to control flight. For example, in the check/stripe condition, bumble-bees fly closer to the stripe pattern in an apparent attempt to balance the optic flow experienced in each eye. We see no such effect in *Megalopta*. Groundspeed also increases in *Megalopta* in the check/stripe condition, even though they are maintaining the same distance from each wall. The differences in behaviour that we observe may be because *Megalopta* use optic flow cues from different parts of the visual field, such as the dorsal or ventral regions, to maintain a safe distance from nearby obstacles and to control groundspeed, or because they have *reduced* their reliance upon vision for flight control in favour
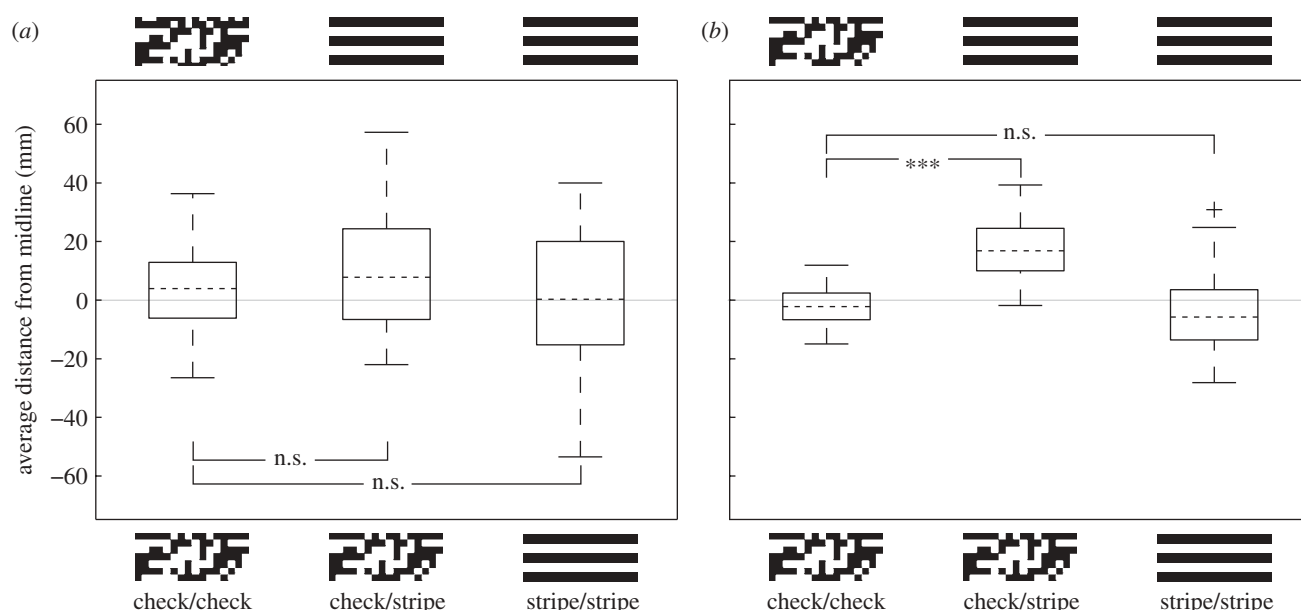
Figure 2. The effect of changes in horizontal optic flow cues on centring in (*a*) *M. genalis* and in (*b*) *B. terrestris*. Thick black lines indicate the tunnel walls; light grey lines indicate the midline of the tunnel and the pattern (check or stripe) indicates the position of the patterns. Other details as in figure 1. In the check/stripe condition, bumble-bees fly closer to the stripe pattern; we see no such effect in *Megalopta*.

of information that is not affected by light intensity, such as mechanosensory measurements of airspeed.

It is interesting to note that, when horizontal optic flow cues are strong, *Megalopta* show more variation in lateral position than bumble-bees. This may be owing to differences in the flight performance of the two species, but another possibility is that the sensory information which *Megalopta* uses to maintain a constant distance between the tunnel walls is noisier or less reliable than the information being used by the bumble-bees.

Overall, the results of this study demonstrate that the visual system of a nocturnal insect is capable of detecting optic flow information in dim light and using it for flight control. This is remarkable considering the sensory challenge of controlling flight in the complex environment of a dark rainforest.

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